

IDK® Zonulin ELISA

**Zur in-vitro-Bestimmung
von Zonulin-Familien-Peptiden (ZFP) in Serum**

**For the in vitro determination of
zonulin family peptides (ZFP) in serum**

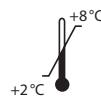
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1. VERWENDUNGSZWECK

Der hier beschriebene Assay ist für die Bestimmung von Zonulin-Familien-Peptiden (ZFP) in Serum geeignet. Nur zur *in-vitro*-Diagnostik.

2. EINLEITUNG

Zonulin ist ein humanes Protein ähnlich dem Zonula-occludens-Toxin von *Vibrio cholerae*, das an der Regulation der interzellulären Kontakte (*tight junctions*) in der Darmwand beteiligt ist. Zonulin bindet an einen spezifischen Rezeptor an der Oberfläche der Epithelzellen der Darmbarriere und aktiviert eine Kaskade biochemischer Ereignisse, welche die Öffnung der *tight junctions* induzieren und als Folge die Durchlässigkeit der Darmepithelzellen erhöhen, so dass verschiedene Substanzen die Darmbarriere passieren und Autoimmunreaktionen auslösen können.

Die Arbeitsgruppe um Fasano hat festgestellt, dass bei Zöliakie- und Typ-1-Diabetes-mellitus-Patienten das Zonulin-System stärker aktiviert ist. Patienten mit aktiver Zöliakie zeigen erhöhte Konzentrationen von Zonulin und Zonulin-Antikörpern im Vergleich zu Nicht-Zöliakiepatienten und Patienten in Remission unter glutenfreier Diät.

Eine erhöhte Darmpermeabilität, die auch unter dem Begriff „Leaky Gut Syndrom“ bekannt ist, wird heutzutage mit dem metabolischen Syndrom, Fettleibigkeit und verschiedenen Autoimmun-, Entzündungs- und Tumorerkrankungen in Verbindung gebracht. Außerdem spielt die erhöhte Darmpermeabilität auch bei Krankheiten wie Multipler Sklerose, rheumatoider Arthritis, Asthma und entzündlichen Darmerkrankungen eine bedeutende Rolle.

Der in diesem ELISA verwendete polyklonale Antikörper basiert auf der Zonulinsequenz, die von Wang (Journal of Cell Science, 2000) und di Pierro (Journal of Biological Chemistry, 2001) publiziert wurde.

Die Messwerte des IDK® Zonulin ELISA, der Zonulin-Familien-Peptide nachweist, korrelieren – wie bereits in vielen Veröffentlichungen festgestellt – mit etablierten metabolischen Merkmalen, die mit einer erhöhten Darmpermeabilität wie Insulinresistenz und Adipositas zusammenhängen.

3. INHALT DER TESTPACKUNG

Art.-Nr.	Bezeichnung	Kit-Komponenten	Menge für Art.-Nr.	
			K 5601	K 5601.20
K 5601	PLATE	Mikrotitermodul, vorbeschichtet	12 x 8 Vertiefungen	20 x 12 x 8 Vertiefungen
K 0001.C.100	WASHBUF	Waschpufferkonzentrat, 10x	2 x 100 ml	40 x 100 ml
K 5601	DIL	Verdünnungspuffer, gebrauchsfertig	1 x 100 ml	20 x 100 ml
K 5601	TRACER	Tracerkonzentrat (biotinyliertes ZFP)	1 x 300 µl	20 x 300 µl
K 5601	CONJ	Konjugatkonzentrat, peroxidasemarkiertes Streptavidin	1 x 200 µl	20 x 200 µl
K 5601	STD	Standards, lyophilisiert (Konzentrationen der Spezifikation entnehmen)	4 x 5 vials	25 x 5 vials
K 5601	CTRL 1	Kontrolle, lyophilisiert (Bereich der Spezifikation entnehmen)	4 x 1 vial	25 x 1 vial
K 5601	CTRL 2	Kontrolle, lyophilisiert (Bereich der Spezifikation entnehmen)	4 x 1 vial	25 x 1 vial
K 0002.15	SUB	Substrat (Tetramethylbenzidin), gebrauchsfertig	1 x 15 ml	20 x 15 ml
K 0003.15	STOP	Stopplösung, gebrauchsfertig	1 x 15 ml	20 x 15 ml

Für Nachbestellungen von Einzelkomponenten verwenden Sie als Bestellnummer die Artikelnummer gefolgt von der Bezeichnung.

4. ERFORDERLICHE LABORGERÄTE UND HILFSMITTEL

- Reinstwasser*
- Präzisionspipetten und Pipettenspitzen für den Einmalgebrauch mit variablen Volumina von 10–1000 µl
- Folie zum Abkleben der Mikrotiterplatte
- Mikrotiterplattenschüttler
- Multikanal- bzw. Multipipette
- Vortex-Mixer
- Laborübliche Glas- oder Plastikrörchen (Einmalartikel) aus Polypropylen
- Mikrotiterplattenphotometer (benötigte Filter siehe Kapitel 7)

* Immundiagnostik AG empfiehlt die Verwendung von Reinstwasser nach ISO 3696. Es handelt sich dabei um Wasser des Typs 1, welches frei von ungelösten und kolloidalen Ionen und organischen Molekülen ist (frei von Partikeln >0,2 µm) mit einer elektrischen Leitfähigkeit von 0,055 µS/cm bei 25 °C ($\geq 18,2 \text{ M}\Omega \text{ cm}$).

5. VORBEREITUNG UND LAGERUNG DER REAGENZIEN

- Bitte achten Sie bei mehrfachem Einsatz des Kits darauf, dass die Reagenzien wie auf dem Etikett angegeben gelagert und **nur die für den jeweiligen Ansatz benötigten Reagenzienmengen frisch angesetzt werden**. Der Kit kann so bis zu 4x je nach Probenaufkommen bis zum angegebenen Haltbarkeitsdatum verwendet werden.
- Reagenzien mit einem **Volumen kleiner 100 µl** sollten vor Gebrauch kurz zentrifugiert werden, um Volumenverluste zu vermeiden.
- **Vorbereitung des Waschpuffers:** Das **Waschpufferkonzentrat (WASHBUF)** muss vor Gebrauch **1:10** in Reinstwasser verdünnt werden (100 ml WASHBUF + 900 ml Reinstwasser), gut mischen. Aufgrund des hohen Salzgehalts im Konzentrat kann es zu Kristallbildungskomplexe kommen. Die Kristalle lösen sich bei Raumtemperatur bzw. im Wasserbad bei 37 °C auf. Das **WASHBUF** kann bei **2–8 °C** bis zum angegebenen Haltbarkeitsdatum aufbewahrt werden. Der **Waschpuffer** (1:10 verdünntes WASHBUF) ist **1 Monat bei 2–8 °C** in einem geschlossenen Gefäß haltbar.
- **Die lyophilisierten Standards (STD) und Kontrollen (CTRL)** sind bei **2–8 °C** bis zum angegebenen Haltbarkeitsdatum verwendbar. Die Rekonstitutionsvorgaben für STD und CTRL sind dem Spezifikationsdatenblatt zu entnehmen. **Standards und Kontrollen** (rekonstituierte STD und CTRL) **sind nicht stabil und können nicht gelagert werden**.

- **Vorbereitung des Tracers:** Das **Tracer-Konzentrat (TRACER)** wird unmittelbar vor Gebrauch **1:101 in Verdünnungspuffer (DIL)** verdünnt (z.B. 150 µl TRACER + 15 ml DIL). Unverdünntes TRACER ist bei **2–8 °C** bis zum angegebenen Haltbarkeitsdatum stabil. **Tracer** (1:101 verdünntes TRACER) **ist nicht stabil und kann nicht aufbewahrt werden.**
- **Vorbereitung des Konjugats:** Das **Konjugatkonzentrat (CONJ)** wird vor Gebrauch **1:101 in Verdünnungspuffer (DIL)** verdünnt (z.B. 100 µl CONJ + 10 ml DIL). Das CONJ ist bei **2–8 °C** bis zum angegebenen Haltbarkeitsdatum stabil. **Konjugat** (1:101 verdünntes CONJ) **ist nicht stabil und kann nicht aufbewahrt werden.**
- Alle anderen Testreagenzien sind gebrauchsfertig und, bei **2–8 °C** gelagert, bis zum angegebenen Verfallsdatum (siehe Etikett) verwendbar.

6. PROBENLAGERUNG UND -VORBEREITUNG

Probenlagerung

ZFP ist in unverdünntem Serum für 12 Monate bei -80 °C sowie für 8 Wochen bei -20 °C und für einen Tag bei 2–8 °C stabil. Bei Raumtemperatur ist ZFP nicht stabil.

Probenverdünnung

1.	Je 25 µl Serumprobe in entsprechend beschriftete Reaktionsgefäße pipettieren.
2.	475 µl Verdünnungspuffer zu jeder Probe zugeben. Dies resultiert in einem Verdünnungsfaktor von 1:20.

7. TESTVORBEREITUNG

Im Test dürfen nur **Reagenzien und Proben** verwendet werden, die **Raumtemperatur** (15–30 °C) aufweisen. Vor Gebrauch Reagenzien und Proben gut mischen.

Markieren Sie die Positionen für Standards/Kontrollen/Proben im Protokollblatt.

Die benötigten Mikrotiterstreifen aus dem Kit nehmen. Nicht verwendete Mikrotiterstreifen müssen zusammen mit dem Trockenmittelbeutel in der verschlossenen Aluminiumverpackung bis zum angegebenen Haltbarkeitsdatum bei 2–8 °C gelagert werden.

Wir empfehlen, die Bestimmungen in Doppelwerten durchzuführen.

Vorbereitung der Standards, Kontrollen und Proben

Je **150 µl Standard, Kontrolle** bzw. **verdünnte Probe** in entsprechend beschriftete Reaktionsgefäße pipettieren und mit **150 µl Tracer** versetzen. Gut vortexen und zeitnah im Test einsetzen.

Wichtig:

Führen Sie die Zugabe des Tracers bei den Standards und Kontrollen gleichzeitig mit verdünnten Proben durch, um deren Gleichbehandlung sicherzustellen.

Standards, Kontrollen und Proben sind nun bereit für den Einsatz im Test.

8. TESTDURCHFÜHRUNG

Testprinzip

Dieser ELISA dient zur quantitativen Bestimmung von ZFP im Serum.

Der Test basiert auf der Methode des kompetitiven ELISA. Die zu untersuchenden Proben, Standards und Kontrollen werden mit einem biotinylierten ZFP-Tracer versetzt und anschließend in einer mit einem polyklonalen anti-ZFP-Antikörper beschichteten ELISA-Platte inkubiert. Während der Inkubation kompetitiert das freie Zielantigen in den Proben mit dem biotinylierten ZFP-Tracer um die Bindung der polyklonalen anti-ZFP-Antikörper. Beim zweiten Inkubationsschritt wird peroxidasemarkiertes Streptavidin zugegeben, das an den biotinylierten ZFP-Tracer bindet. Nach einem Waschschritt zur Entfernung ungebundener Komponenten wird das Peroxidasesubstrat Tetramethylbenzidin (TMB) zugegeben. Die Enzymreaktion wird durch Zugabe von Säure abgestoppt. Dadurch erfolgt ein Farbumschlag von blau nach gelb. Die entstandene chromogene Verbindung wird photometrisch bei 450 nm gemessen. Die Intensität der Farbe ist umgekehrt proportional zur Konzentration des gemessenen Analyten, d. h. mit steigender ZFP-Konzentration in der Probe reduziert sich die Konzentration des an den anti-ZFP-Antikörper gebundenen biotinylierten ZFP-Tracers und das Signal nimmt ab. Anhand einer mitgeführten Standardkurve – optische Dichte (Absorption bei 450 nm) versus Standardkonzentration – lässt sich die Konzentration der Probe ermitteln.

Pipettierschema

Im Fall einer automatisierten Abarbeitung des Tests können automaten spezifische Anpassungen der Prozedur notwendig sein, um den jeweiligen technischen Gegebenheiten gerecht zu werden. Für Unterstützung und Rückfragen wenden Sie sich bitte an Ihren Anbieter oder Immundiagnostik AG.

1.	100 µl der vorbereiteten Standards/Kontrollen/Proben in die jeweiligen Vertiefungen pipettieren.
2.	Streifen abdecken und 1 Stunde bei Raumtemperatur (15–30 °C) unter Schütteln bei 550 Upm mit einem Orbit von 2 mm inkubieren.
3.	Inhalt der Vertiefungen verwerfen und 5x mit je 250 µl Waschpuffer waschen. Nach dem letzten Waschschnitt Reste von Waschpuffer durch Ausklopfen auf saugfähigem Papier entfernen.
4.	100 µl Konjugat (verdünntes CONJ) in jede Vertiefung pipettieren.
5.	Streifen abdecken und 1 Stunde bei Raumtemperatur (15–30 °C) unter Schütteln bei 550 Upm mit einem Orbit von 2 mm inkubieren.
6.	Inhalt der Vertiefungen verwerfen und 5x mit je 250 µl Waschpuffer waschen. Nach dem letzten Waschschnitt Reste von Waschpuffer durch Ausklopfen auf saugfähigem Papier entfernen.
7.	100 µl Substrat (SUB) in jede Vertiefung pipettieren.
8.	10–20 Minuten bei Raumtemperatur (15–30 °C) im Dunkeln inkubieren*.
9.	100 µl Stopplösung (STOP) in jede Vertiefung pipettieren, gut mischen.
10.	Extinktion sofort im Mikrotiterplattenphotometer bei 450 nm gegen die Referenzwellenlänge 620 nm (oder 690 nm) messen. Ist keine Referenzwellenlänge vorhanden, wird nur bei 450 nm gemessen. Falls die Extinktion einer Probe oder eines Standards den Messbereich des Photometers übersteigt, sollte sofort bei 405 nm gegen 620 nm (690 nm) gemessen werden.

* Die Intensität der Farbentwicklung ist temperaturabhängig. Es wird empfohlen den Farbumschlag während der Inkubationszeit zu beobachten und entsprechend der Farbentwicklung die Reaktion zu stoppen.

9. ERGEBNISSE

Die unten beschriebenen mathematischen Modelle können alternativ zur Auswertung benutzt werden. Wir empfehlen die 4-Parameter-Funktion:

1. 4-Parameter-Funktion

Für die optische Dichte empfehlen wir eine lineare Ordinate und für die Konzentration eine logarithmische Abszisse (bei einer logarithmischen Abszisse muss für den Standard mit der Konzentration 0 ein Wert kleiner 1 eingegeben werden z.B. 0,001).

2. Punkt-zu-Punkt-Auswertung

Für die optische Dichte und für die Konzentration empfehlen wir eine lineare Ordinate bzw. Abszisse.

3. Gewichtete Spline-Funktion

Für die optische Dichte und für die Konzentration empfehlen wir eine lineare Ordinate bzw. Abszisse.

Vor jeder automatischen Auswertung sollte stets eine Kontrolle der Doppelwerte auf Plausibilität („Ausreißerkontrolle“) durchgeführt werden; falls dies nicht durch das verwendete Programm erfolgt, sollte die Kontrolle manuell durchgeführt werden.

Serumproben

Die ermittelten Ergebnisse werden mit dem **Verdünnungsfaktor 20** multipliziert, um die tatsächlichen Konzentrationen zu erhalten.

Sollte ein **anderer Verdünnungsfaktor** verwendet worden sein, so ist die ermittelte Konzentration mit dem verwendeten Verdünnungsfaktor zu multiplizieren.

10. EINSCHRÄNKUNGEN

Proben mit Konzentrationen oberhalb des Messbereichs (Definition siehe unten) können stärker verdünnt und erneut gemessen werden. Bitte beachten Sie diese stärkere Verdünnung bei der Ergebnisberechnung.

Proben mit Konzentrationen unterhalb des Messbereichs (Definition siehe unten) können nicht klar quantifiziert werden.

Die Obergrenze des Messbereichs ergibt sich aus:

höchste Konzentration der Standardkurve × anzuwendender Probenverdünnungsfaktor

Die Untergrenze des Messbereichs ergibt sich aus:

LoB × anzuwendender Probenverdünnungsfaktor

LoB siehe Kapitel „Testcharakteristika“.

11. QUALITÄTSKONTROLLE

Immundiagnostik AG empfiehlt den Einsatz von externen Kontrollen für die interne Qualitätskontrolle, wenn möglich.

Wir empfehlen, bei jedem Testansatz Kontrollen mitzumessen. Die Ergebnisse der Kontrollen müssen auf Richtigkeit überprüft werden. Liegen eine oder mehrere Kontrollen außerhalb des angegebenen Bereiches, kann Immundiagnostik AG die Richtigkeit der Messergebnisse nicht gewährleisten.

Referenzwerte

Anhand einer laborinternen Studie mit Serumproben von augenscheinlich Gesunden ($n = 40$) wurde ein Medianwert von 34 ng/ml (± 14 ng/ml) ermittelt.

Wir empfehlen jedem Labor, einen eigenen Referenzbereich zu etablieren.

Für Plasmaproben sind eigene Referenzbereiche zu erheben.

12. TESTCHARAKTERISTIKA

Genauigkeit – Präzision

Wiederholbarkeit (Intra-Assay); n=40

Die Wiederholbarkeit wurde mit 2 Serumproben unter gleichbleibenden Bedingungen (Bediener, System, Tag, Kitcharge) bestimmt.

Probe	Mittelwert [ng/ml]	VK [%]
1	43,90	3,5
2	38,38	6,0

Reproduzierbarkeit (Inter-Assay); n=25

Die Reproduzierbarkeit wurde mit 2 Serumproben unter variablen Bedingungen (Bediener, System, Tag, Kitcharge) bestimmt.

Probe	Mittelwert [ng/ml]	VK [%]
1	41,13	7,7
2	46,15	8,3

Genauigkeit – Richtigkeit

Die Richtigkeit gibt das Verhältnis zwischen dem Messergebnis und der wahren Konzentration einer Probe an. 3 Serumproben wurden dafür mit bekannten ZFP-Konzentrationen versetzt und gemessen.

Probe [ng/ml]	Spike [ng/ml]	Erwartet [ng/ml]	Gemessen [ng/ml]	Wieder- findung [%]
10,788	51,58	62,469	66,331	106,2
	37,71	48,494	50,259	103,6
	26,64	37,430	34,469	92,1
12,428	51,58	64,109	70,666	110,2
	37,71	50,134	50,883	101,5
	26,64	39,070	33,032	84,5
13,372	51,58	65,053	70,547	108,4
	37,71	51,078	53,548	104,8
	26,64	40,014	34,266	85,6

Linearität

Die Linearität zeigt die Fähigkeit einer Methode, ein Ergebnis proportional zur Analytkonzentration in einer Probe zu liefern. Sie wurde gemäß CLSI-Richtlinie EP6-A mittels einer seriellen Verdünnung zweier Serumproben nachgewiesen.

Für ZFP in Serum wurde ein lineares Verhalten im Bereich von 3,03 bis 40,25 ng/ml nachgewiesen. Die Nicht-Linearität lag bei weniger als $\pm 20\%$.

Probe	Verdünnung	Erwartet [ng/ml]	Gemessen [ng/ml]	Wieder- findung [%]
A	1:20	36,69	36,69	100,00
	1:40	18,34	19,02	103,70
	1:80	9,17	9,92	108,17
	1:160	4,59	5,91	128,79
B	1:20	40,25	40,25	100,00
	1:40	20,13	20,41	101,41
	1:80	10,06	10,90	108,29
	1:160	5,03	5,64	112,09
	1:320	2,52	3,03	120,52

Analytische Sensitivität

Die im Folgenden aufgeführten Werte wurden in Bezug auf die Standardkurve ohne Berücksichtigung eventuell verwendeter Probenverdünnungsfaktoren ermittelt.

Leerwert (<i>limit of blank</i> , LoB)	0,140 ng/ml
Nachweisgrenze (<i>limit of detection</i> , LoD)	0,183 ng/ml
Bestimmungsgrenze (<i>limit of quantitation</i> , LoQ)	0,183 ng/ml

Die Auswertung wurde gemäß der CLSI-Richtlinie EP17-A2 durchgeführt. Das festgelegte Präzisionsziel für die Bestimmungsgrenze lag bei 20 % VK.

Analytische Spezifität

Die Spezifität wurde nachgewiesen durch Bestimmung der Kreuzreakтивität zu humanem Haptoglobin. Es wurde keine Kreuzreakтивität nachgewiesen.

Getestete Substanz	Eingesetzte Konzentration	Gefundene Konzentration	Fazit
Humanes Haptoglobin	2,9 mg/ml	< 0,02 ng/ml	< LoB

13. VORSICHTSMASSNAHMEN

- Alle im Kit enthaltenen Reagenzien dürfen ausschließlich zur *in-vitro*-Diagnostik verwendet werden.
- Das für Kitkomponenten verwendete humane Material wurde auf HIV, Hepatitis B und Hepatitis C getestet und für negativ befunden. Dennoch wird empfohlen, die Kitkomponenten als Vorsichtsmaßnahme immer wie potentiell infektiöses Material zu behandeln.
- Die Kitkomponenten enthalten zum Schutz vor bakteriellen Kontaminationen Natriumazid oder ProClin. Natriumazid bzw. ProClin sind giftig. Auch Substrate für enzymatische Farbreaktionen sind als giftig und karzinogen beschrieben. Jeder Kontakt mit Haut oder Schleimhaut ist zu vermeiden.
- Die Stopplösung besteht aus verdünnter Schwefelsäure (H_2SO_4). H_2SO_4 ist eine starke Säure und muss auch in verdünnter Form mit Vorsicht benutzt werden. H_2SO_4 verursacht bei Kontakt mit der Haut Verätzungen. Es sollte daher mit Schutzhandschuhen, Schutzkleidung und Schutzbrille gearbeitet werden. Bei Kontakt mit der Säure muss die verätzte Stelle sofort mit viel Wasser gespült werden. Dämpfe nicht einatmen und Inhalation vermeiden.

14. TECHNISCHE MERKMALE

- Reagenzien der Testpackung dürfen nicht mit anderen Chargen gemischt werden. Ferner dürfen Kavitäten unterschiedlicher Mikrotiterplatten, selbst der gleichen Charge, nicht zusammengefügt und zur Analyse verwendet werden.
- Qualitätskontrollen sollten immer mitgemessen werden.
- Die Reagenzien dürfen nach Ablauf des auf der Kitverpackung angegebenen Haltbarkeitsdatums nicht mehr verwendet werden.
- Substratlösung muss vor Gebrauch farblos sein.
- Mikrotiterstreifen müssen während der Inkubationen mit Folie abgedeckt sein.
- Vermeiden Sie Schaumbildung beim Mischen der Reagenzien.
- Stopfen und Verschlüsse verschiedener Reagenzien dürfen nicht vertauscht werden.
- Der Assay ist immer nach der im Kit beigefügten Arbeitsanleitung durchzuführen.

15. ALLGEMEINE HINWEISE ZUM TEST

- Dieser Kit wurde nach der IVD-Richtlinie 98/79/EG hergestellt und in den Verkehr gebracht.
- Für die Qualitätskontrolle sind die für medizinische Laboratorien erstellten Richtlinien zu beachten.
- *IDK*® ist eine Marke der Immundiagnostik AG.
- Die Testcharakteristika wie Inkubationszeiten, Inkubationstemperaturen und Pipettievolumina der verschiedenen Komponenten wurden vom Hersteller festgelegt. Nicht mit dem Hersteller abgesprochene Veränderungen in der Testdurchführung können die Resultate beeinflussen. Die Firma Immundiagnostik AG übernimmt für die hierdurch entstandenen Schäden und Folgeschäden keine Haftung.
- Bei Gewährleistungsansprüchen ist das beanstandete Material mit schriftlicher Erklärung innerhalb von 14 Tagen zum Hersteller, der Immundiagnostik AG, zurück zu senden.

16. LITERATUR

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® Zonulin ELISA

***For the in vitro determination of
zonulin family peptides (ZFP) in serum***

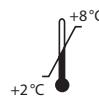
Valid from 2019-05-07



K 5601



K 5601.20



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1. INTENDED USE

This ELISA is intended for the quantitative determination of zonulin family peptides (ZFP) in serum. For *in vitro* diagnostic use only.

2. INTRODUCTION

Zonulin is a human protein analogue to the zonula occludens toxin derived from *Vibrio cholerae* which regulates tight junctions of the digestive tract. Zonulin binds to a specific receptor on the surface of intestinal epithelia and triggers a cascade of biochemical events which induces tight junction disassembly and a subsequent permeability increase of the intestinal epithelia, allowing some substances to pass through and activate immune reactions.

Fasano and his co-workers found that the zonulin system is more activated in celiac disease and type 1 diabetes mellitus patients. Patients with active celiac disease showed higher levels of zonulin and anti-zonulin antibodies compared to non-celiac patients and patients in remission, who were on a gluten-free diet.

An increased intestinal permeability, also colloquially called 'leaky gut', is nowadays associated with the metabolic syndrome, obesity, and several autoimmune, inflammatory, and neoplastic diseases. Based on evidence, leaky gut plays a meaningful role in diseases such as multiple sclerosis, rheumatoid arthritis, asthma, and inflammatory bowel diseases.

The polyclonal anti-body used in our ELISA is based on the zonulin sequence as published by Wang (Journal of Cell Science, 2000) and di Pierro (Journal of Biological Chemistry, 2001).

Correspondingly, the readings of IDK® Zonulin ELISA detecting zonulin family peptides correlate well - as already found in many papers - with established metabolic traits linked to increased gut permeability, such as insulin resistance and obesity.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity for cat. no.	
			K 5601	K 5601.20
K 5601	PLATE	Microtiter plate, pre-coated	12 x 8 wells	20 x 12 x 8 wells
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10x	2 x 100 ml	40 x 100 ml

Cat. No.	Label	Kit components	Quantity for cat. no.	
			K 5601	K 5601.20
K 5601	DIL	Dilution buffer, ready-to-use	1 x 100 ml	20 x 100 ml
K 5601	TRACER	Tracer concentrate, biotinylated ZFP	1 x 300 µl	20 x 300 µl
K 5601	CONJ	Conjugate concentrate, peroxidase-labelled streptavidin	1 x 200 µl	20 x 200 µl
K 5601	STD	Standards, lyophilised (see specification for concentrations)	4 x 5 vials	25 x 5 vials
K 5601	CTRL1	Control, lyophilised (see specification for range)	4 x 1 vial	25 x 1 vial
K 5601	CTRL2	Control, ready-to-use (see specification for range)	4 x 1 vial	25 x 1 vial
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml	20 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml	20 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles >0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C ($\geq 18.2 \text{ M}\Omega \text{ cm}$).

5. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a **volume less than 100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month.**
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet. Standards and controls** (reconstituted STD and CTRL) **are not stable and cannot be stored.**
- **Preparation of the tracer:** The **tracer concentrate (TRACER)** has to be diluted **1:101 in dilution buffer** (e.g. 150 µl TRACER + 15 ml DIL) immediately before use. The TRACER is stable at **2–8 °C** until expiry date given on the label. **Tracer** (1:101 diluted TRACER) **is not stable and cannot be stored.**
- **Preparation of the conjugate:** Immediately before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in dilution buffer (e.g. 100 µl CONJ + 10 ml DIL). The CONJ is stable at **2–8 °C** until expiry date stated on the label. **Conjugate** (1:101 diluted CONJ) **is not stable and cannot be stored.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C.**

6. STORAGE AND PREPARATION OF SAMPLES

Sample Storage

ZFP is stable in undiluted serum for 12 months at -80 °C as well as for 8 weeks at -20 °C and for 1 day at 2–8 °C. ZFP is not stable at room temperature.

Sample dilution

- | | |
|----|--|
| 1. | Pipet each 25 µl of serum samples in the respectively labelled reaction tubes. |
| 2. | Add 475 µl of dilution buffer to each sample. This results in a dilution factor of 20. |

7. PREPARATION OF THE ASSAY

Prior to use, allow all reagents and samples to come to room temperature (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

We recommend to carry out the tests in duplicate.

Preparation of standards, controls and diluted samples

Transfer **150 µl** of each **standard, control or diluted sample** in the correspondingly labelled reaction tubes and add **150 µl** of **tracer**. Vortex well and use promptly in the test.

Important:

Carry out the addition of tracer simultaneously with standards, controls and diluted samples in order to ensure equal treatment.

Standards, controls and samples are now ready for use in the test.

8. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of ZFP in serum samples.

This assay is based on the method of competitive ELISA. As a first preparation step, a biotinylated ZFP tracer is added to the samples, standards and controls. Afterwards, aliquots of the treated samples, standards and controls are transferred and incubated in microtiter plate wells coated with polyclonal anti-ZFP antibodies. During the incubation, the free target antigen in the samples competes with the biotinylated ZFP tracer for the binding of the polyclonal anti-ZFP antibodies immobilised on the microtiter plate wells. The unbound components are removed by a washing step.

During a second incubation step, peroxidase-labelled streptavidin, which binds to the biotinylated ZFP tracer, is added into each microtiter well. After a washing step to remove the unbound components, the peroxidase substrate tetramethylbenzidine (TMB) is added. Finally, the enzymatic reaction is terminated by an acidic stop solution. The colour changes from blue to yellow and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow colour is inverse proportional to the ZFP concentration in the sample; this means, high ZFP concentration in the sample reduces the concentration of the biotinylated ZFP tracer bound to the immobilised anti-ZFP antibodies and lowers the photometric signal. A dose response curve of absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. ZFP, present in the patient samples, is determined directly from this curve.

Test procedure

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

1.	Add each 100 µl of the prepared standards/controls/samples into the respective wells.
2.	Cover the strips and incubate for 1 hour shaking on a horizontal shaker at 550 rpm with an orbit of 2 mm at room temperature (15–30 °C).
3.	Discard the content of each well and wash 5 times with 250 µl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
4.	Add 100 µl conjugate (diluted CONJ) into each well.
5.	Cover the strips and incubate for 1 hour shaking on a horizontal shaker at 550 rpm with an orbit of 2 mm at room temperature (15–30 °C).
6.	Discard the content of each well and wash 5 times with 250 µl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
7.	Add 100 µl substrate (SUB) into each well.
8.	Incubate for 10–20 minutes at room temperature (15–30 °C)* in the dark .
9.	Add 100 µl stop solution (STOP) into each well and mix well.

10. Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of a sample or a standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm as a reference.

* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

9. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum samples

The obtained results have to be multiplied by the **dilution factor of 20** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

10. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement (see definition below) range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

LoB see chapter "Performance characteristics".

11. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

Based on Immundiagnostik AG studies of serum samples of apparently healthy persons ($n = 40$), a median value of 34 ng/ml (± 14 ng/ml) was estimated.

We recommend each laboratory to establish its own reference range.

Establish own reference ranges for plasma samples.

12. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n=40

The repeatability was assessed with 2 serum samples under constant parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [ng/ml]	CV [%]
1	43.90	3.5
2	38.38	6.0

Reproducibility (Inter-Assay); n=25

The reproducibility was assessed with 2 serum samples under varying parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [ng/ml]	CV [%]
1	41.13	7.7
2	46.15	8.3

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, ZFP spikes with known concentrations were added to 3 different serum samples.

Sample [ng/ml]	Spike [ng/ml]	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
10.788	51.58	62.469	66.331	106.2
	37.71	48.494	50.259	103.6
	26.64	37.430	34.469	92.1
12.428	51.58	64.109	70.666	110.2
	37.71	50.134	50.883	101.5
	26.64	39.070	33.032	84.5
13.372	51.58	65.053	70.547	108.4
	37.71	51.078	53.548	104.8
	26.64	40.014	34.266	85.6

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline EP6-A with a serial dilution of 2 different serum samples.

For ZFP in serum, the method has been demonstrated to be linear from 3.03–40.25 ng/ml, showing a non-linear behaviour of less than $\pm 20\%$ in this interval for concentrations greater than the Limit of Quantitation.

Sample	Dilution	Expected [ng/ml]	Obtained [ng/ml]	Recovery [%]
A	1:20	36.69	36.69	100.00
	1:40	18.34	19.02	103.70
	1:80	9.17	9.92	108.17
	1:160	4.59	5.91	128.79
B	1:20	40.25	40.25	100.00
	1:40	20.13	20.41	101.41
	1:80	10.06	10.90	108.29
	1:160	5.03	5.64	112.09
	1:320	2.52	3.03	120.52

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors

Limit of blank, LoB 0.140 ng/ml

Limit of detection, LoD 0.183 ng/ml

Limit of quantitation, LoQ 0.183 ng/ml

The evaluation was performed according to the CLSI guideline EP17-A2. The specified accuracy goal for the LoQ was 20% CV.

Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against human haptoglobin. There was no cross-reactivity observed.

Substance tested	Concentration added	Concentration obtained	Conclusion
Human haptoglobin	2.9 mg/ml	< 0.02 ng/ml	< LoB

13. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Kit reagents contain sodium azide or Proclin as bactericides. Sodium azide and Proclin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

14. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

15. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- IDK® is a trademark of Immundiagnostik AG.

- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

16. REFERENCES

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Used symbols:



Temperature limitation

REF

Catalogue Number

IVD

In Vitro Diagnostic Medical Device

→REF

To be used with



Manufacturer



Contains sufficient for <n> tests

LOT

Lot number



Use by



Attention



Consult instructions for use



Consult specification data sheet



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Supplementary Material

Supplement 1. The randomization scheme of the exercise protocols for all volunteers An individual sequence of exercise protocols was assigned to each volunteer.

P1	P2	P3	P5	P4
P1	P3	P2	P5	P4
P1	P5	P2	P3	P4
P1	P4	P5	P3	P2
P1	P4	P2	P3	P5
P1	P5	P4	P3	P2
P1	P3	P2	P4	P5
P1	P4	P5	P2	P3
P1	P5	P3	P2	P4
P1	P4	P3	P2	P5
P1	P5	P4	P2	P3
P1	P2	P3	P5	P4
P1	P2	P3	P4	P5
P1	P3	P2	P5	P4
P1	P5	P4	P2	-

Supplementary Material

Supplement 2. The abbreviations with the corresponding full names of the 34 parameters which are listed in the heatmap (Figure 3).

Nr	Abbreviation parameter	Full name parameter
1	lymph	lymphocytes
2	citrul	citrulline
3	bicar	bicarbonate
4	tyr	tyrosine
5	ala	alanine
6	pota	potassium
7	neut	neutrophils
8	cort	cortisol
9	creat	creatinine
10	hb	hemoglobin
11	ht	hematocrite
12	eryt	erythrocytes
13	ifabp	intestinal fatty acid binding protein
14	mono	monocytes
15	phe	phenylalanine
16	albu	albumine
17	trp	tryptophan
18	chlo	chloride
19	sodi	sodium
20	tau	taurine
21	thr	threonine
22	gln	glutamine
23	asn	asparagine
24	leu	leucine
25	ser	serine
26	arg	arginine
27	ile	isoleucine
28	val	valine
29	urea	urea
30	glu	glutamic acid
31	lys	lysine
32	gly	glycine
33	asp	asparic acid
34	meth	methionine

Supplementary Material

Supplement 3. An overview of the data in this publication. The data and the graphs included are also available as an R-package at

https://uashogeschoolutrecht.github.io/kinetics_tables.html

protocol	time	analyte	mean	sd	n_obs
P1	0.0	ala	341.338	80.73	15
P1	0.5	ala	366.255	89.81	15
P1	1.0	ala	386.725	84.74	15
P1	1.5	ala	992.711	308.07	15
P1	2.0	ala	799.408	215.06	14
P1	24.0	ala	393.618	69.22	15
P1	3.0	ala	466.513	125.38	15
P1	6.0	ala	335.779	75.26	15
P2	0.0	ala	357.909	42.42	15
P2	0.5	ala	616.712	46.59	14
P2	1.0	ala	668.840	66.56	15
P2	1.5	ala	1208.113	277.14	15
P2	2.0	ala	900.982	189.79	14
P2	24.0	ala	355.717	69.83	15
P2	3.0	ala	462.063	94.60	15
P2	6.0	ala	320.843	52.00	14
P3	0.0	ala	357.735	72.24	14
P3	0.5	ala	603.730	77.93	13
P3	1.0	ala	604.195	118.97	13
P3	1.5	ala	754.698	182.47	14
P3	2.0	ala	942.173	242.25	12
P3	24.0	ala	375.976	59.46	14
P3	3.0	ala	496.984	140.82	13
P3	6.0	ala	311.157	48.98	12
P4	0.0	ala	377.211	77.99	15
P4	0.5	ala	491.294	115.44	14

protocol	time	analyte	mean	sd	n_obs
P4	1.0	ala	481.333	98.03	15
P4	1.5	ala	1141.309	238.92	15
P4	2.0	ala	758.676	126.61	14
P4	24.0	ala	356.489	52.00	15
P4	3.0	ala	450.945	95.02	14
P4	6.0	ala	311.806	57.92	14
P5	0.0	ala	356.556	85.42	15
P5	0.5	ala	635.664	100.83	15
P5	1.0	ala	660.353	145.20	15
P5	1.5	ala	1238.774	284.32	14
P5	2.0	ala	986.901	234.98	14
P5	24.0	ala	346.989	77.57	15
P5	3.0	ala	456.491	118.24	15
P5	6.0	ala	304.783	64.42	15
P1	0.0	ALB	44.400	1.96	15
P1	0.5	ALB	44.533	2.50	15
P1	1.0	ALB	44.467	3.14	15
P1	1.5	ALB	45.071	2.90	14
P1	2.0	ALB	45.333	2.74	15
P1	24.0	ALB	44.667	2.19	15
P1	3.0	ALB	44.733	2.87	15
P1	6.0	ALB	45.200	2.83	15
P2	0.0	ALB	43.867	1.85	15
P2	0.5	ALB	48.462	2.33	13
P2	1.0	ALB	47.867	2.39	15
P2	1.5	ALB	45.733	2.28	15
P2	2.0	ALB	44.286	2.43	14
P2	24.0	ALB	44.267	2.22	15
P2	3.0	ALB	44.533	2.33	15
P2	6.0	ALB	45.571	2.62	14
P3	0.0	ALB	44.714	2.23	14
P3	0.5	ALB	49.769	2.46	13
P3	1.0	ALB	49.000	2.83	14
P3	1.5	ALB	46.643	2.59	14

protocol	time	analyte	mean	sd	n_obs
P3	2.0	ALB	45.333	2.60	12
P3	24.0	ALB	44.643	2.40	14
P3	3.0	ALB	45.500	2.14	14
P3	6.0	ALB	46.000	2.76	11
P4	0.0	ALB	43.667	2.16	15
P4	0.5	ALB	47.357	2.40	14
P4	1.0	ALB	46.933	1.94	15
P4	1.5	ALB	44.933	2.68	15
P4	2.0	ALB	43.929	1.94	14
P4	24.0	ALB	44.625	1.93	16
P4	3.0	ALB	44.667	1.92	15
P4	6.0	ALB	44.929	2.64	14
P5	0.0	ALB	43.600	1.64	15
P5	0.5	ALB	49.133	1.96	15
P5	1.0	ALB	48.667	2.58	15
P5	1.5	ALB	44.929	1.73	14
P5	2.0	ALB	44.214	2.19	14
P5	24.0	ALB	43.714	2.84	14
P5	3.0	ALB	44.533	2.72	15
P5	6.0	ALB	45.667	2.35	15
P1	0.0	arg	94.777	12.34	15
P1	0.5	arg	116.724	22.02	15
P1	1.0	arg	114.290	13.82	15
P1	1.5	arg	110.013	15.20	15
P1	2.0	arg	109.196	13.85	14
P1	24.0	arg	102.215	11.03	15
P1	3.0	arg	93.574	14.30	15
P1	6.0	arg	86.079	10.84	15
P2	0.0	arg	99.258	10.58	15
P2	0.5	arg	116.921	15.44	14
P2	1.0	arg	123.293	12.56	15
P2	1.5	arg	117.168	15.30	15
P2	2.0	arg	112.362	15.13	14
P2	24.0	arg	95.259	8.33	15

protocol	time	analyte	mean	sd	n_obs
P2	3.0	arg	89.491	10.79	15
P2	6.0	arg	85.446	9.23	14
P3	0.0	arg	99.448	15.13	14
P3	0.5	arg	108.671	13.53	13
P3	1.0	arg	109.286	17.59	13
P3	1.5	arg	113.196	16.69	14
P3	2.0	arg	114.766	17.65	12
P3	24.0	arg	95.241	10.27	14
P3	3.0	arg	96.844	9.17	13
P3	6.0	arg	83.952	8.93	12
P4	0.0	arg	96.891	13.37	15
P4	0.5	arg	129.740	20.38	14
P4	1.0	arg	128.451	16.05	15
P4	1.5	arg	115.941	17.99	15
P4	2.0	arg	109.979	16.58	14
P4	24.0	arg	96.909	10.91	15
P4	3.0	arg	95.002	9.33	14
P4	6.0	arg	87.806	7.75	14
P5	0.0	arg	97.241	10.03	15
P5	0.5	arg	115.469	14.92	15
P5	1.0	arg	120.703	16.27	15
P5	1.5	arg	114.650	14.35	14
P5	2.0	arg	113.706	12.70	14
P5	24.0	arg	92.571	9.78	15
P5	3.0	arg	88.768	11.19	15
P5	6.0	arg	84.219	8.21	15
P1	0.0	asn	50.573	7.76	15
P1	0.5	asn	59.560	10.35	15
P1	1.0	asn	59.236	9.22	15
P1	1.5	asn	76.997	14.73	15
P1	2.0	asn	65.957	13.69	14
P1	24.0	asn	55.401	7.04	15
P1	3.0	asn	49.099	8.88	15
P1	6.0	asn	46.966	7.13	15

protocol	time	analyte	mean	sd	n_obs
P2	0.0	asn	52.814	6.80	15
P2	0.5	asn	56.534	9.50	14
P2	1.0	asn	59.397	10.13	15
P2	1.5	asn	72.675	13.50	15
P2	2.0	asn	65.674	15.04	14
P2	24.0	asn	55.207	5.91	15
P2	3.0	asn	43.841	8.04	15
P2	6.0	asn	45.481	6.56	14
P3	0.0	asn	49.114	8.46	14
P3	0.5	asn	49.409	8.16	13
P3	1.0	asn	51.440	9.08	13
P3	1.5	asn	57.337	10.32	14
P3	2.0	asn	62.197	11.51	12
P3	24.0	asn	54.651	6.79	14
P3	3.0	asn	44.385	10.23	13
P3	6.0	asn	42.151	7.36	12
P4	0.0	asn	51.144	7.25	15
P4	0.5	asn	62.441	11.88	14
P4	1.0	asn	60.943	8.97	15
P4	1.5	asn	73.609	14.43	15
P4	2.0	asn	60.074	12.66	14
P4	24.0	asn	52.297	6.81	15
P4	3.0	asn	46.027	7.41	14
P4	6.0	asn	44.988	6.32	14
P5	0.0	asn	51.724	6.25	15
P5	0.5	asn	55.467	8.71	15
P5	1.0	asn	57.335	8.50	15
P5	1.5	asn	72.266	11.86	14
P5	2.0	asn	67.682	10.48	14
P5	24.0	asn	54.508	6.70	15
P5	3.0	asn	43.197	7.36	15
P5	6.0	asn	44.097	6.63	15
P1	0.0	asp	5.275	1.63	15
P1	0.5	asp	6.070	3.25	15

protocol	time	analyte	mean	sd	n_obs
P1	1.0	asp	5.351	1.90	15
P1	1.5	asp	8.700	2.86	15
P1	2.0	asp	7.145	2.63	14
P1	24.0	asp	6.852	5.39	15
P1	3.0	asp	5.747	1.65	15
P1	6.0	asp	4.785	1.32	15
P2	0.0	asp	5.891	1.84	15
P2	0.5	asp	6.416	1.24	14
P2	1.0	asp	6.936	2.04	15
P2	1.5	asp	10.136	3.48	15
P2	2.0	asp	7.716	2.30	14
P2	24.0	asp	5.481	1.70	15
P2	3.0	asp	5.039	1.43	15
P2	6.0	asp	4.664	1.33	14
P3	0.0	asp	6.212	2.89	14
P3	0.5	asp	5.854	0.97	13
P3	1.0	asp	6.389	1.19	13
P3	1.5	asp	8.299	2.45	14
P3	2.0	asp	8.001	1.64	12
P3	24.0	asp	5.405	1.61	14
P3	3.0	asp	5.719	1.45	13
P3	6.0	asp	4.253	0.92	12
P4	0.0	asp	6.015	1.66	15
P4	0.5	asp	6.559	1.45	14
P4	1.0	asp	7.563	1.69	15
P4	1.5	asp	10.565	2.84	15
P4	2.0	asp	7.849	1.55	14
P4	24.0	asp	5.763	1.02	15
P4	3.0	asp	6.553	3.06	14
P4	6.0	asp	4.731	0.90	14
P5	0.0	asp	5.314	1.26	15
P5	0.5	asp	6.282	1.37	15
P5	1.0	asp	6.295	0.96	15
P5	1.5	asp	12.082	7.02	14

protocol	time	analyte	mean	sd	n_obs
P5	2.0	asp	8.126	1.64	14
P5	24.0	asp	5.179	1.05	15
P5	3.0	asp	4.989	1.36	15
P5	6.0	asp	4.428	1.03	15
P1	0.0	BICARB	25.067	1.53	15
P1	0.5	BICARB	27.133	2.72	15
P1	1.0	BICARB	27.267	3.04	15
P1	1.5	BICARB	26.214	2.01	14
P1	2.0	BICARB	28.667	4.48	15
P1	24.0	BICARB	26.267	1.62	15
P1	3.0	BICARB	27.200	2.34	15
P1	6.0	BICARB	26.333	2.94	15
P2	0.0	BICARB	25.467	1.77	15
P2	0.5	BICARB	18.357	1.74	14
P2	1.0	BICARB	19.400	2.64	15
P2	1.5	BICARB	22.933	2.31	15
P2	2.0	BICARB	24.571	2.71	14
P2	24.0	BICARB	25.200	1.61	15
P2	3.0	BICARB	24.800	1.66	15
P2	6.0	BICARB	24.214	1.97	14
P3	0.0	BICARB	25.357	1.22	14
P3	0.5	BICARB	17.385	2.10	13
P3	1.0	BICARB	19.857	3.57	14
P3	1.5	BICARB	24.357	2.62	14
P3	2.0	BICARB	24.500	3.78	12
P3	24.0	BICARB	25.500	1.45	14
P3	3.0	BICARB	26.000	4.37	14
P3	6.0	BICARB	24.818	1.78	11
P4	0.0	BICARB	25.467	1.36	15
P4	0.5	BICARB	23.929	1.94	14
P4	1.0	BICARB	23.600	2.10	15
P4	1.5	BICARB	23.800	2.57	15
P4	2.0	BICARB	24.929	1.68	14
P4	24.0	BICARB	26.438	1.63	16

protocol	time	analyte	mean	sd	n_obs
P4	3.0	BICARB	25.400	1.68	15
P4	6.0	BICARB	26.000	1.71	14
P5	0.0	BICARB	25.067	2.19	15
P5	0.5	BICARB	17.467	3.34	15
P5	1.0	BICARB	18.067	3.83	15
P5	1.5	BICARB	22.786	2.75	14
P5	2.0	BICARB	23.857	2.41	14
P5	24.0	BICARB	25.643	2.13	14
P5	3.0	BICARB	24.667	2.32	15
P5	6.0	BICARB	24.733	2.12	15
P1	0.0	CHLORIDE	100.067	3.17	15
P1	0.5	CHLORIDE	100.267	2.79	15
P1	1.0	CHLORIDE	100.133	3.23	15
P1	1.5	CHLORIDE	99.571	3.06	14
P1	2.0	CHLORIDE	100.267	3.04	15
P1	24.0	CHLORIDE	99.467	2.17	15
P1	3.0	CHLORIDE	100.067	2.68	15
P1	6.0	CHLORIDE	98.800	2.54	15
P2	0.0	CHLORIDE	99.733	1.58	15
P2	0.5	CHLORIDE	99.538	1.98	13
P2	1.0	CHLORIDE	99.067	2.31	15
P2	1.5	CHLORIDE	98.133	1.88	15
P2	2.0	CHLORIDE	98.571	2.28	14
P2	24.0	CHLORIDE	100.200	1.82	15
P2	3.0	CHLORIDE	96.933	1.83	15
P2	6.0	CHLORIDE	96.571	2.03	14
P3	0.0	CHLORIDE	100.643	2.68	14
P3	0.5	CHLORIDE	100.692	2.14	13
P3	1.0	CHLORIDE	100.643	2.37	14
P3	1.5	CHLORIDE	101.500	2.25	14
P3	2.0	CHLORIDE	101.750	2.42	12
P3	24.0	CHLORIDE	99.643	2.44	14
P3	3.0	CHLORIDE	100.286	1.82	14
P3	6.0	CHLORIDE	97.091	2.26	11

protocol	time	analyte	mean	sd	n_obs
P4	0.0	CHLORIDE	99.600	1.76	15
P4	0.5	CHLORIDE	101.429	2.31	14
P4	1.0	CHLORIDE	99.867	2.07	15
P4	1.5	CHLORIDE	99.000	2.56	15
P4	2.0	CHLORIDE	98.643	2.06	14
P4	24.0	CHLORIDE	100.125	2.90	16
P4	3.0	CHLORIDE	98.333	2.06	15
P4	6.0	CHLORIDE	97.286	2.34	14
P5	0.0	CHLORIDE	100.467	2.36	15
P5	0.5	CHLORIDE	100.467	2.07	15
P5	1.0	CHLORIDE	99.733	1.98	15
P5	1.5	CHLORIDE	98.286	2.09	14
P5	2.0	CHLORIDE	98.071	2.06	14
P5	24.0	CHLORIDE	101.143	2.51	14
P5	3.0	CHLORIDE	98.000	2.20	15
P5	6.0	CHLORIDE	97.400	2.47	15
P1	0.0	citrul	32.917	6.48	15
P1	0.5	citrul	30.921	5.17	15
P1	1.0	citrul	28.970	5.82	15
P1	1.5	citrul	32.128	6.17	15
P1	2.0	citrul	41.649	7.34	14
P1	24.0	citrul	35.491	6.03	15
P1	3.0	citrul	34.669	7.45	15
P1	6.0	citrul	31.099	5.74	15
P2	0.0	citrul	34.735	5.48	15
P2	0.5	citrul	37.791	5.72	14
P2	1.0	citrul	42.616	6.38	15
P2	1.5	citrul	43.933	7.34	15
P2	2.0	citrul	46.157	9.35	14
P2	24.0	citrul	37.303	6.49	15
P2	3.0	citrul	35.389	6.91	15
P2	6.0	citrul	31.222	5.17	14
P3	0.0	citrul	36.100	7.41	14
P3	0.5	citrul	34.555	8.05	13

protocol	time	analyte	mean	sd	n_obs
P3	1.0	citrul	33.455	8.31	13
P3	1.5	citrul	36.759	8.35	14
P3	2.0	citrul	38.453	8.34	12
P3	24.0	citrul	37.434	6.93	14
P3	3.0	citrul	35.455	8.21	13
P3	6.0	citrul	32.857	6.66	12
P4	0.0	citrul	34.881	5.62	15
P4	0.5	citrul	39.144	7.65	14
P4	1.0	citrul	43.833	10.18	15
P4	1.5	citrul	44.041	9.62	15
P4	2.0	citrul	50.536	9.80	14
P4	24.0	citrul	36.596	7.04	15
P4	3.0	citrul	39.016	7.69	14
P4	6.0	citrul	32.331	5.22	14
P5	0.0	citrul	35.125	5.97	15
P5	0.5	citrul	37.366	6.90	15
P5	1.0	citrul	40.739	5.92	15
P5	1.5	citrul	41.354	6.44	14
P5	2.0	citrul	46.314	8.15	14
P5	24.0	citrul	37.275	6.42	15
P5	3.0	citrul	34.209	7.48	15
P5	6.0	citrul	30.746	5.12	15
P1	0.0	CORT	0.548	0.12	15
P1	0.5	CORT	0.462	0.12	15
P1	1.0	CORT	0.422	0.11	14
P1	1.5	CORT	0.346	0.08	14
P1	2.0	CORT	0.312	0.07	15
P1	24.0	CORT	0.480	0.10	15
P1	3.0	CORT	0.275	0.06	15
P1	6.0	CORT	0.283	0.08	15
P2	0.0	CORT	0.450	0.09	15
P2	0.5	CORT	0.536	0.11	14
P2	1.0	CORT	0.663	0.14	15
P2	1.5	CORT	0.638	0.17	15

protocol	time	analyte	mean	sd	n_obs
P2	2.0	CORT	0.558	0.15	14
P2	24.0	CORT	0.414	0.08	15
P2	3.0	CORT	0.419	0.11	15
P2	6.0	CORT	0.184	0.07	14
P3	0.0	CORT	0.515	0.09	14
P3	0.5	CORT	0.554	0.09	13
P3	1.0	CORT	0.725	0.08	14
P3	1.5	CORT	0.687	0.10	14
P3	2.0	CORT	0.569	0.07	12
P3	24.0	CORT	0.436	0.08	14
P3	3.0	CORT	0.463	0.11	14
P3	6.0	CORT	0.180	0.07	11
P4	0.0	CORT	0.483	0.08	15
P4	0.5	CORT	0.433	0.10	14
P4	1.0	CORT	0.382	0.11	15
P4	1.5	CORT	0.308	0.08	15
P4	2.0	CORT	0.282	0.08	14
P4	24.0	CORT	0.460	0.07	15
P4	3.0	CORT	0.252	0.07	15
P4	6.0	CORT	0.253	0.11	14
P5	0.0	CORT	0.500	0.10	15
P5	0.5	CORT	0.509	0.10	15
P5	1.0	CORT	0.645	0.15	15
P5	1.5	CORT	0.584	0.13	14
P5	2.0	CORT	0.484	0.13	14
P5	24.0	CORT	0.437	0.08	14
P5	3.0	CORT	0.369	0.12	15
P5	6.0	CORT	0.249	0.12	15
P1	0.0	ERY	4.893	0.32	15
P1	0.5	ERY	4.907	0.34	14
P1	1.0	ERY	4.867	0.33	15
P1	1.5	ERY	4.907	0.32	15
P1	2.0	ERY	4.880	0.35	15
P1	24.0	ERY	4.920	0.33	15

protocol	time	analyte	mean	sd	n_obs
P1	3.0	ERY	4.847	0.33	15
P1	6.0	ERY	4.847	0.31	15
P2	0.0	ERY	4.750	0.35	14
P2	0.5	ERY	5.092	0.34	13
P2	1.0	ERY	5.050	0.39	14
P2	1.5	ERY	4.757	0.38	14
P2	2.0	ERY	4.662	0.37	13
P2	24.0	ERY	4.620	0.30	15
P2	3.0	ERY	4.714	0.34	14
P2	6.0	ERY	4.708	0.36	13
P3	0.0	ERY	4.685	0.21	13
P3	0.5	ERY	5.092	0.22	12
P3	1.0	ERY	4.954	0.20	13
P3	1.5	ERY	4.769	0.21	13
P3	2.0	ERY	4.667	0.24	12
P3	24.0	ERY	4.529	0.20	14
P3	3.0	ERY	4.662	0.20	13
P3	6.0	ERY	4.570	0.28	10
P4	0.0	ERY	4.657	0.26	14
P4	0.5	ERY	4.977	0.28	13
P4	1.0	ERY	4.893	0.29	14
P4	1.5	ERY	4.650	0.24	14
P4	2.0	ERY	4.623	0.25	13
P4	24.0	ERY	4.719	0.30	16
P4	3.0	ERY	4.650	0.26	14
P4	6.0	ERY	4.608	0.29	13
P5	0.0	ERY	4.687	0.30	15
P5	0.5	ERY	5.173	0.38	15
P5	1.0	ERY	5.047	0.36	15
P5	1.5	ERY	4.713	0.33	15
P5	2.0	ERY	4.640	0.36	15
P5	24.0	ERY	4.614	0.33	14
P5	3.0	ERY	4.627	0.35	15
P5	6.0	ERY	4.700	0.38	15

protocol	time	analyte	mean	sd	n_obs
P1	0.0	gln	581.327	41.06	15
P1	0.5	gln	607.819	50.26	15
P1	1.0	gln	635.520	51.06	15
P1	1.5	gln	957.102	120.57	15
P1	2.0	gln	899.379	133.35	14
P1	24.0	gln	587.312	59.46	15
P1	3.0	gln	624.139	87.38	15
P1	6.0	gln	584.750	48.82	15
P2	0.0	gln	564.054	48.92	15
P2	0.5	gln	575.913	40.10	14
P2	1.0	gln	604.669	40.99	15
P2	1.5	gln	885.463	125.02	15
P2	2.0	gln	799.978	119.71	14
P2	24.0	gln	568.093	43.44	15
P2	3.0	gln	569.228	79.89	15
P2	6.0	gln	542.419	71.05	14
P3	0.0	gln	577.851	41.61	14
P3	0.5	gln	608.334	52.13	13
P3	1.0	gln	618.808	66.79	13
P3	1.5	gln	732.213	91.33	14
P3	2.0	gln	873.640	124.87	12
P3	24.0	gln	563.329	49.35	14
P3	3.0	gln	627.805	80.64	13
P3	6.0	gln	558.720	55.79	12
P4	0.0	gln	553.805	44.72	15
P4	0.5	gln	561.657	63.07	14
P4	1.0	gln	559.068	50.88	15
P4	1.5	gln	971.819	126.28	15
P4	2.0	gln	783.109	103.14	14
P4	24.0	gln	553.690	47.43	15
P4	3.0	gln	575.264	62.14	14
P4	6.0	gln	546.764	43.18	14
P5	0.0	gln	561.694	43.22	15
P5	0.5	gln	564.484	61.74	15

protocol	time	analyte	mean	sd	n_obs
P5	1.0	gln	594.787	75.85	15
P5	1.5	gln	914.552	119.93	14
P5	2.0	gln	848.400	81.51	14
P5	24.0	gln	573.009	33.05	15
P5	3.0	gln	561.343	67.86	15
P5	6.0	gln	552.849	43.08	15
P1	0.0	glu	97.305	36.12	15
P1	0.5	glu	95.527	31.84	15
P1	1.0	glu	80.728	29.52	15
P1	1.5	glu	126.321	36.70	15
P1	2.0	glu	107.578	24.50	14
P1	24.0	glu	104.043	50.39	15
P1	3.0	glu	91.206	22.55	15
P1	6.0	glu	90.983	32.40	15
P2	0.0	glu	114.600	27.50	15
P2	0.5	glu	152.009	39.57	14
P2	1.0	glu	162.585	49.18	15
P2	1.5	glu	190.165	54.80	15
P2	2.0	glu	159.269	50.05	14
P2	24.0	glu	112.403	35.13	15
P2	3.0	glu	105.056	33.45	15
P2	6.0	glu	118.071	41.98	14
P3	0.0	glu	101.094	23.02	14
P3	0.5	glu	128.048	42.45	13
P3	1.0	glu	144.425	36.28	13
P3	1.5	glu	158.046	35.12	14
P3	2.0	glu	151.291	35.37	12
P3	24.0	glu	101.384	22.80	14
P3	3.0	glu	111.579	24.49	13
P3	6.0	glu	101.293	34.44	12
P4	0.0	glu	117.364	25.28	15
P4	0.5	glu	120.456	26.59	14
P4	1.0	glu	136.082	33.23	15
P4	1.5	glu	183.801	38.73	15

protocol	time	analyte	mean	sd	n_obs
P4	2.0	glu	148.316	16.94	14
P4	24.0	glu	115.703	25.76	15
P4	3.0	glu	108.353	28.84	14
P4	6.0	glu	105.541	22.72	14
P5	0.0	glu	105.805	19.14	15
P5	0.5	glu	148.115	46.44	15
P5	1.0	glu	142.530	39.29	15
P5	1.5	glu	192.751	44.27	14
P5	2.0	glu	149.515	23.29	14
P5	24.0	glu	101.995	14.65	15
P5	3.0	glu	102.535	32.57	15
P5	6.0	glu	107.033	32.41	15
P1	0.0	GLU_NS	5.167	0.34	15
P1	1.0	GLU_NS	4.387	0.58	15
P1	2.0	GLU_NS	4.613	0.37	15
P1	6.0	GLU_NS	4.873	0.29	15
P2	0.0	GLU_NS	5.067	0.24	15
P2	1.0	GLU_NS	5.480	0.82	15
P2	2.0	GLU_NS	4.643	0.37	14
P2	6.0	GLU_NS	4.714	0.25	14
P3	0.0	GLU_NS	5.064	0.34	14
P3	1.0	GLU_NS	5.643	1.07	14
P3	2.0	GLU_NS	5.042	0.43	12
P3	6.0	GLU_NS	4.791	0.30	11
P4	0.0	GLU_NS	4.993	0.30	15
P4	1.0	GLU_NS	4.853	0.35	15
P4	2.0	GLU_NS	4.871	0.20	14
P4	6.0	GLU_NS	4.750	0.21	14
P5	0.0	GLU_NS	4.980	0.31	15
P5	1.0	GLU_NS	5.293	1.14	15
P5	2.0	GLU_NS	4.453	0.38	15
P5	6.0	GLU_NS	4.593	0.34	15
P1	0.0	gly	271.173	125.74	15
P1	0.5	gly	269.307	69.58	15

protocol	time	analyte	mean	sd	n_obs
P1	1.0	gly	265.551	51.94	15
P1	1.5	gly	294.417	51.11	15
P1	2.0	gly	261.634	47.17	14
P1	24.0	gly	258.019	80.62	15
P1	3.0	gly	218.632	38.36	15
P1	6.0	gly	213.843	29.18	15
P2	0.0	gly	250.699	46.30	15
P2	0.5	gly	263.844	47.10	14
P2	1.0	gly	272.938	47.58	15
P2	1.5	gly	280.343	65.21	15
P2	2.0	gly	260.611	71.24	14
P2	24.0	gly	227.395	30.47	15
P2	3.0	gly	197.243	40.22	15
P2	6.0	gly	198.733	37.63	14
P3	0.0	gly	245.292	27.73	14
P3	0.5	gly	255.975	29.71	13
P3	1.0	gly	260.328	31.88	13
P3	1.5	gly	246.046	29.98	14
P3	2.0	gly	249.961	24.53	12
P3	24.0	gly	233.429	25.45	14
P3	3.0	gly	201.654	29.63	13
P3	6.0	gly	194.250	24.68	12
P4	0.0	gly	247.385	23.37	15
P4	0.5	gly	271.816	29.98	14
P4	1.0	gly	277.421	31.70	15
P4	1.5	gly	282.663	39.75	15
P4	2.0	gly	244.104	39.27	14
P4	24.0	gly	230.345	32.48	15
P4	3.0	gly	210.064	27.25	14
P4	6.0	gly	203.537	28.42	14
P5	0.0	gly	244.253	36.33	15
P5	0.5	gly	258.062	37.16	15
P5	1.0	gly	262.043	37.23	15
P5	1.5	gly	274.325	49.69	14

protocol	time	analyte	mean	sd	n_obs
P5	2.0	gly	267.431	43.53	14
P5	24.0	gly	216.579	32.92	15
P5	3.0	gly	191.897	34.64	15
P5	6.0	gly	189.237	36.58	15
P1	0.0	HB	9.227	0.48	15
P1	0.5	HB	9.236	0.54	14
P1	1.0	HB	9.187	0.57	15
P1	1.5	HB	9.220	0.58	15
P1	2.0	HB	9.207	0.61	15
P1	24.0	HB	9.233	0.56	15
P1	3.0	HB	9.140	0.56	15
P1	6.0	HB	9.147	0.50	15
P2	0.0	HB	8.836	0.55	14
P2	0.5	HB	9.615	0.52	13
P2	1.0	HB	9.493	0.54	14
P2	1.5	HB	8.950	0.53	14
P2	2.0	HB	8.769	0.53	13
P2	24.0	HB	8.733	0.45	15
P2	3.0	HB	8.807	0.51	14
P2	6.0	HB	8.823	0.54	13
P3	0.0	HB	8.831	0.41	13
P3	0.5	HB	9.600	0.44	12
P3	1.0	HB	9.423	0.47	13
P3	1.5	HB	9.031	0.44	13
P3	2.0	HB	8.750	0.44	12
P3	24.0	HB	8.564	0.40	14
P3	3.0	HB	8.815	0.39	13
P3	6.0	HB	8.670	0.38	10
P4	0.0	HB	8.779	0.50	14
P4	0.5	HB	9.392	0.54	13
P4	1.0	HB	9.279	0.56	14
P4	1.5	HB	8.807	0.44	14
P4	2.0	HB	8.739	0.47	13
P4	24.0	HB	8.853	0.49	15

protocol	time	analyte	mean	sd	n_obs
P4	3.0	HB	8.793	0.52	14
P4	6.0	HB	8.731	0.58	13
P5	0.0	HB	8.773	0.57	15
P5	0.5	HB	9.660	0.50	15
P5	1.0	HB	9.453	0.51	15
P5	1.5	HB	8.853	0.48	15
P5	2.0	HB	8.713	0.51	15
P5	24.0	HB	8.671	0.55	14
P5	3.0	HB	8.707	0.53	15
P5	6.0	HB	8.780	0.57	15
P1	0.0	HT	0.416	0.02	15
P1	0.5	HT	0.415	0.02	14
P1	1.0	HT	0.417	0.02	15
P1	1.5	HT	0.419	0.03	15
P1	2.0	HT	0.416	0.03	15
P1	24.0	HT	0.419	0.02	15
P1	3.0	HT	0.413	0.03	15
P1	6.0	HT	0.411	0.02	15
P2	0.0	HT	0.402	0.02	14
P2	0.5	HT	0.438	0.02	13
P2	1.0	HT	0.432	0.02	14
P2	1.5	HT	0.403	0.02	14
P2	2.0	HT	0.395	0.03	13
P2	24.0	HT	0.399	0.02	15
P2	3.0	HT	0.398	0.02	14
P2	6.0	HT	0.398	0.03	13
P3	0.0	HT	0.405	0.02	13
P3	0.5	HT	0.441	0.02	12
P3	1.0	HT	0.429	0.02	13
P3	1.5	HT	0.410	0.02	13
P3	2.0	HT	0.401	0.02	12
P3	24.0	HT	0.390	0.02	14
P3	3.0	HT	0.400	0.02	13
P3	6.0	HT	0.389	0.02	10

protocol	time	analyte	mean	sd	n_obs
P4	0.0	HT	0.401	0.02	14
P4	0.5	HT	0.425	0.02	13
P4	1.0	HT	0.420	0.02	14
P4	1.5	HT	0.399	0.02	14
P4	2.0	HT	0.398	0.02	13
P4	24.0	HT	0.402	0.02	16
P4	3.0	HT	0.400	0.02	14
P4	6.0	HT	0.395	0.02	13
P5	0.0	HT	0.400	0.02	15
P5	0.5	HT	0.445	0.03	15
P5	1.0	HT	0.436	0.02	15
P5	1.5	HT	0.402	0.02	15
P5	2.0	HT	0.395	0.02	15
P5	24.0	HT	0.399	0.02	14
P5	3.0	HT	0.393	0.02	15
P5	6.0	HT	0.398	0.03	15
P1	0.0	ifabp	841.374	448.27	15
P1	0.5	ifabp	855.446	440.44	15
P1	1.0	ifabp	642.700	344.38	15
P1	1.5	ifabp	435.986	269.16	14
P1	2.0	ifabp	415.095	306.21	15
P1	24.0	ifabp	830.411	455.76	15
P1	3.0	ifabp	679.362	427.39	15
P1	6.0	ifabp	643.611	400.08	15
P2	0.0	ifabp	742.860	341.13	15
P2	0.5	ifabp	910.381	270.55	14
P2	1.0	ifabp	1262.841	512.74	14
P2	1.5	ifabp	1098.044	585.32	15
P2	2.0	ifabp	788.346	448.48	14
P2	24.0	ifabp	816.947	381.08	14
P2	3.0	ifabp	468.897	292.74	15
P2	6.0	ifabp	505.601	250.98	14
P3	0.0	ifabp	689.776	309.97	14
P3	0.5	ifabp	917.689	349.19	14

protocol	time	analyte	mean	sd	n_obs
P3	1.0	ifabp	1559.869	658.93	14
P3	1.5	ifabp	1443.720	901.23	13
P3	2.0	ifabp	956.983	699.57	12
P3	24.0	ifabp	900.535	563.24	14
P3	3.0	ifabp	448.870	252.01	14
P3	6.0	ifabp	537.664	328.94	12
P4	0.0	ifabp	698.842	369.10	15
P4	0.5	ifabp	700.636	377.15	13
P4	1.0	ifabp	598.556	360.26	15
P4	1.5	ifabp	387.107	204.16	15
P4	2.0	ifabp	346.737	164.66	14
P4	24.0	ifabp	646.478	367.54	10
P4	3.0	ifabp	505.551	300.57	15
P4	6.0	ifabp	583.784	275.07	14
P5	0.0	ifabp	720.480	359.21	15
P5	0.5	ifabp	901.460	402.89	14
P5	1.0	ifabp	1198.754	714.66	15
P5	1.5	ifabp	807.915	513.93	14
P5	2.0	ifabp	535.861	306.07	14
P5	24.0	ifabp	745.058	360.87	14
P5	3.0	ifabp	424.613	243.32	15
P5	6.0	ifabp	511.252	324.59	15
P1	0.0	ile	64.292	8.93	15
P1	0.5	ile	73.086	12.16	15
P1	1.0	ile	70.355	9.45	15
P1	1.5	ile	62.755	8.41	15
P1	2.0	ile	54.145	9.67	14
P1	24.0	ile	75.578	9.19	15
P1	3.0	ile	53.579	7.63	15
P1	6.0	ile	64.315	5.79	15
P2	0.0	ile	70.783	9.11	15
P2	0.5	ile	79.640	8.16	14
P2	1.0	ile	83.923	12.38	15
P2	1.5	ile	72.600	10.99	15

protocol	time	analyte	mean	sd	n_obs
P2	2.0	ile	62.432	11.13	14
P2	24.0	ile	78.295	12.31	15
P2	3.0	ile	56.784	8.03	15
P2	6.0	ile	77.864	11.53	14
P3	0.0	ile	70.399	11.59	14
P3	0.5	ile	75.305	12.19	13
P3	1.0	ile	75.907	13.72	13
P3	1.5	ile	75.050	11.52	14
P3	2.0	ile	69.097	11.99	12
P3	24.0	ile	76.672	12.17	14
P3	3.0	ile	56.326	8.27	13
P3	6.0	ile	72.263	8.64	12
P4	0.0	ile	67.935	10.84	15
P4	0.5	ile	83.227	13.86	14
P4	1.0	ile	81.524	12.82	15
P4	1.5	ile	66.907	11.81	15
P4	2.0	ile	57.249	12.11	14
P4	24.0	ile	75.625	11.97	15
P4	3.0	ile	53.668	11.03	14
P4	6.0	ile	69.044	8.50	14
P5	0.0	ile	71.243	9.64	15
P5	0.5	ile	79.369	10.23	15
P5	1.0	ile	81.933	10.11	15
P5	1.5	ile	73.488	11.93	14
P5	2.0	ile	63.816	11.13	14
P5	24.0	ile	82.261	12.46	15
P5	3.0	ile	62.049	17.79	15
P5	6.0	ile	83.115	21.79	15
P1	0.0	INSULINE	5.920	1.54	15
P1	0.5	INSULINE	24.460	11.31	15
P1	1.0	INSULINE	10.240	4.37	15
P1	1.5	INSULINE	11.350	5.09	14
P1	2.0	INSULINE	7.213	2.34	15
P1	24.0	INSULINE	6.540	1.64	15

protocol	time	analyte	mean	sd	n_obs
P1	3.0	INSULINE	4.693	1.93	15
P1	6.0	INSULINE	3.460	1.03	15
P2	0.0	INSULINE	5.620	2.23	15
P2	0.5	INSULINE	3.643	1.82	14
P2	1.0	INSULINE	3.420	2.60	15
P2	1.5	INSULINE	13.280	5.75	15
P2	2.0	INSULINE	6.464	2.97	14
P2	24.0	INSULINE	5.133	1.22	15
P2	3.0	INSULINE	4.167	2.45	15
P2	6.0	INSULINE	2.771	1.01	14
P3	0.0	INSULINE	6.036	1.82	14
P3	0.5	INSULINE	3.815	1.97	13
P3	1.0	INSULINE	3.329	2.71	14
P3	1.5	INSULINE	20.293	10.91	14
P3	2.0	INSULINE	13.250	4.21	12
P3	24.0	INSULINE	5.550	1.71	14
P3	3.0	INSULINE	5.779	1.98	14
P3	6.0	INSULINE	3.027	1.11	11
P4	0.0	INSULINE	6.673	2.56	15
P4	0.5	INSULINE	8.500	3.57	14
P4	1.0	INSULINE	3.700	2.07	15
P4	1.5	INSULINE	11.507	5.79	15
P4	2.0	INSULINE	7.050	3.12	14
P4	24.0	INSULINE	5.213	2.12	16
P4	3.0	INSULINE	4.720	1.84	15
P4	6.0	INSULINE	2.986	1.05	14
P5	0.0	INSULINE	6.253	2.68	15
P5	0.5	INSULINE	5.080	2.49	15
P5	1.0	INSULINE	3.800	3.58	15
P5	1.5	INSULINE	12.786	9.81	14
P5	2.0	INSULINE	6.107	4.28	14
P5	24.0	INSULINE	6.300	1.90	14
P5	3.0	INSULINE	3.100	1.90	15
P5	6.0	INSULINE	3.013	1.46	15

protocol	time	analyte	mean	sd	n_obs
P1	0.0	KREAT	81.600	11.49	15
P1	0.5	KREAT	79.600	9.44	15
P1	1.0	KREAT	79.600	10.01	15
P1	1.5	KREAT	77.143	9.44	14
P1	2.0	KREAT	77.533	10.34	15
P1	24.0	KREAT	79.933	10.10	15
P1	3.0	KREAT	75.333	9.74	15
P1	6.0	KREAT	74.267	8.40	15
P2	0.0	KREAT	81.600	9.43	15
P2	0.5	KREAT	93.357	10.94	14
P2	1.0	KREAT	103.533	15.04	15
P2	1.5	KREAT	97.533	11.95	15
P2	2.0	KREAT	94.500	8.33	14
P2	24.0	KREAT	82.800	9.02	15
P2	3.0	KREAT	87.533	10.16	15
P2	6.0	KREAT	81.643	8.72	14
P3	0.0	KREAT	84.214	9.33	14
P3	0.5	KREAT	98.385	12.83	13
P3	1.0	KREAT	107.571	17.93	14
P3	1.5	KREAT	97.357	14.63	14
P3	2.0	KREAT	95.917	13.81	12
P3	24.0	KREAT	82.857	8.15	14
P3	3.0	KREAT	91.714	11.36	14
P3	6.0	KREAT	83.727	12.27	11
P4	0.0	KREAT	83.467	9.65	15
P4	0.5	KREAT	85.643	11.15	14
P4	1.0	KREAT	85.733	12.13	15
P4	1.5	KREAT	83.867	11.88	15
P4	2.0	KREAT	83.714	11.25	14
P4	24.0	KREAT	81.750	8.87	16
P4	3.0	KREAT	81.933	11.44	15
P4	6.0	KREAT	79.000	9.77	14
P5	0.0	KREAT	82.133	9.23	15
P5	0.5	KREAT	91.400	13.03	15

protocol	time	analyte	mean	sd	n_obs
P5	1.0	KREAT	101.667	16.93	15
P5	1.5	KREAT	94.143	13.24	14
P5	2.0	KREAT	91.929	11.92	14
P5	24.0	KREAT	83.286	8.20	14
P5	3.0	KREAT	86.600	10.51	15
P5	6.0	KREAT	80.600	10.34	15
P1	0.0	leu	122.333	12.56	15
P1	0.5	leu	136.405	19.66	15
P1	1.0	leu	130.520	12.34	15
P1	1.5	leu	117.849	10.96	15
P1	2.0	leu	101.465	13.59	14
P1	24.0	leu	130.289	11.50	15
P1	3.0	leu	99.190	10.37	15
P1	6.0	leu	122.911	13.03	15
P2	0.0	leu	125.771	11.39	15
P2	0.5	leu	140.815	12.09	14
P2	1.0	leu	149.446	16.92	15
P2	1.5	leu	131.596	16.55	15
P2	2.0	leu	113.485	17.00	14
P2	24.0	leu	131.262	16.41	15
P2	3.0	leu	100.072	13.24	15
P2	6.0	leu	133.068	15.86	14
P3	0.0	leu	124.434	15.34	14
P3	0.5	leu	133.461	15.13	13
P3	1.0	leu	136.811	18.68	13
P3	1.5	leu	137.367	16.97	14
P3	2.0	leu	124.579	18.55	12
P3	24.0	leu	126.958	14.09	14
P3	3.0	leu	98.988	11.96	13
P3	6.0	leu	122.147	11.56	12
P4	0.0	leu	123.935	15.30	15
P4	0.5	leu	148.069	16.84	14
P4	1.0	leu	144.186	16.87	15
P4	1.5	leu	120.520	15.60	15

protocol	time	analyte	mean	sd	n_obs
P4	2.0	leu	101.729	14.95	14
P4	24.0	leu	129.824	17.15	15
P4	3.0	leu	93.206	13.25	14
P4	6.0	leu	121.796	11.48	14
P5	0.0	leu	127.034	16.42	15
P5	0.5	leu	140.112	17.03	15
P5	1.0	leu	145.542	16.01	15
P5	1.5	leu	132.894	20.80	14
P5	2.0	leu	112.756	20.82	14
P5	24.0	leu	134.416	17.03	15
P5	3.0	leu	106.799	31.70	15
P5	6.0	leu	140.962	41.02	15
P1	0.0	LEUCO	5.253	0.84	15
P1	0.5	LEUCO	5.064	0.80	14
P1	1.0	LEUCO	5.547	1.13	15
P1	1.5	LEUCO	5.787	1.07	15
P1	2.0	LEUCO	6.013	1.19	15
P1	24.0	LEUCO	5.207	0.90	15
P1	3.0	LEUCO	5.993	1.22	15
P1	6.0	LEUCO	6.200	1.14	15
P2	0.0	LEUCO	5.021	1.02	14
P2	0.5	LEUCO	9.323	2.01	13
P2	1.0	LEUCO	9.600	2.21	14
P2	1.5	LEUCO	7.186	2.05	14
P2	2.0	LEUCO	9.000	2.27	13
P2	24.0	LEUCO	4.787	1.14	15
P2	3.0	LEUCO	11.386	2.23	14
P2	6.0	LEUCO	9.331	1.88	13
P3	0.0	LEUCO	4.962	1.02	13
P3	0.5	LEUCO	9.300	1.69	12
P3	1.0	LEUCO	9.185	1.99	13
P3	1.5	LEUCO	7.392	1.89	13
P3	2.0	LEUCO	9.583	2.60	12
P3	24.0	LEUCO	5.007	0.96	14

protocol	time	analyte	mean	sd	n_obs
P3	3.0	LEUCO	11.292	2.44	13
P3	6.0	LEUCO	9.000	1.76	10
P4	0.0	LEUCO	4.836	0.76	14
P4	0.5	LEUCO	7.362	1.02	13
P4	1.0	LEUCO	7.786	1.36	14
P4	1.5	LEUCO	5.536	0.87	14
P4	2.0	LEUCO	6.431	1.12	13
P4	24.0	LEUCO	4.781	1.12	16
P4	3.0	LEUCO	7.521	1.40	14
P4	6.0	LEUCO	6.808	1.34	13
P5	0.0	LEUCO	4.707	0.65	15
P5	0.5	LEUCO	9.420	1.40	15
P5	1.0	LEUCO	9.380	1.48	15
P5	1.5	LEUCO	7.227	3.42	15
P5	2.0	LEUCO	9.873	4.32	15
P5	24.0	LEUCO	4.936	0.91	14
P5	3.0	LEUCO	11.953	3.06	15
P5	6.0	LEUCO	9.807	2.20	15
P1	0.0	LYMFO_ABS_CORR	1.960	0.50	15
P1	0.5	LYMFO_ABS_CORR	1.650	0.44	14
P1	1.0	LYMFO_ABS_CORR	1.653	0.43	15
P1	1.5	LYMFO_ABS_CORR	1.633	0.45	15
P1	2.0	LYMFO_ABS_CORR	1.720	0.46	15
P1	24.0	LYMFO_ABS_CORR	1.847	0.47	15
P1	3.0	LYMFO_ABS_CORR	1.721	0.46	14
P1	6.0	LYMFO_ABS_CORR	2.087	0.56	15
P2	0.0	LYMFO_ABS_CORR	1.993	0.43	14
P2	0.5	LYMFO_ABS_CORR	4.093	1.14	14
P2	1.0	LYMFO_ABS_CORR	4.307	1.24	15
P2	1.5	LYMFO_ABS_CORR	1.473	0.43	15
P2	2.0	LYMFO_ABS_CORR	1.321	0.36	14
P2	24.0	LYMFO_ABS_CORR	1.787	0.51	15
P2	3.0	LYMFO_ABS_CORR	1.350	0.40	14
P2	6.0	LYMFO_ABS_CORR	2.062	0.62	13

protocol	time	analyte	mean	sd	n_obs
P3	0.0	LYMFO_ABS_CORR	1.857	0.63	14
P3	0.5	LYMFO_ABS_CORR	4.046	1.28	13
P3	1.0	LYMFO_ABS_CORR	3.679	1.27	14
P3	1.5	LYMFO_ABS_CORR	1.393	0.39	14
P3	2.0	LYMFO_ABS_CORR	1.200	0.40	13
P3	24.0	LYMFO_ABS_CORR	1.871	0.58	14
P3	3.0	LYMFO_ABS_CORR	1.250	0.42	14
P3	6.0	LYMFO_ABS_CORR	1.818	0.67	11
P4	0.0	LYMFO_ABS_CORR	1.813	0.47	15
P4	0.5	LYMFO_ABS_CORR	2.736	0.74	14
P4	1.0	LYMFO_ABS_CORR	2.720	0.84	15
P4	1.5	LYMFO_ABS_CORR	1.533	0.45	15
P4	2.0	LYMFO_ABS_CORR	1.629	0.54	14
P4	24.0	LYMFO_ABS_CORR	1.819	0.56	16
P4	3.0	LYMFO_ABS_CORR	1.853	0.53	15
P4	6.0	LYMFO_ABS_CORR	2.121	0.66	14
P5	0.0	LYMFO_ABS_CORR	1.860	0.42	15
P5	0.5	LYMFO_ABS_CORR	4.400	1.01	15
P5	1.0	LYMFO_ABS_CORR	4.233	1.19	15
P5	1.5	LYMFO_ABS_CORR	1.507	0.70	15
P5	2.0	LYMFO_ABS_CORR	1.436	0.72	14
P5	24.0	LYMFO_ABS_CORR	1.843	0.50	14
P5	3.0	LYMFO_ABS_CORR	1.367	0.36	15
P5	6.0	LYMFO_ABS_CORR	1.900	0.45	14
P1	0.0	lys	152.277	32.49	15
P1	0.5	lys	161.801	35.48	15
P1	1.0	lys	158.131	27.39	15
P1	1.5	lys	156.690	30.46	15
P1	2.0	lys	150.622	28.42	14
P1	24.0	lys	162.783	26.27	15
P1	3.0	lys	136.151	22.04	15
P1	6.0	lys	140.955	23.97	15
P2	0.0	lys	156.515	29.70	15
P2	0.5	lys	170.249	30.00	14

protocol	time	analyte	mean	sd	n_obs
P2	1.0	lys	173.588	30.16	15
P2	1.5	lys	154.339	26.32	15
P2	2.0	lys	150.647	28.95	14
P2	24.0	lys	154.519	22.76	15
P2	3.0	lys	125.530	23.05	15
P2	6.0	lys	136.037	18.75	14
P3	0.0	lys	154.567	30.42	14
P3	0.5	lys	163.730	28.86	13
P3	1.0	lys	165.697	35.42	13
P3	1.5	lys	153.881	28.89	14
P3	2.0	lys	144.929	32.23	12
P3	24.0	lys	150.247	26.14	14
P3	3.0	lys	124.646	20.92	13
P3	6.0	lys	125.573	20.35	12
P4	0.0	lys	155.045	28.92	15
P4	0.5	lys	170.804	28.05	14
P4	1.0	lys	167.423	28.98	15
P4	1.5	lys	154.503	27.38	15
P4	2.0	lys	147.427	26.67	14
P4	24.0	lys	157.897	26.00	15
P4	3.0	lys	129.151	21.18	14
P4	6.0	lys	137.047	15.54	14
P5	0.0	lys	153.394	26.59	15
P5	0.5	lys	163.511	27.67	15
P5	1.0	lys	167.381	26.75	15
P5	1.5	lys	149.930	25.54	14
P5	2.0	lys	147.046	29.00	14
P5	24.0	lys	154.286	24.32	15
P5	3.0	lys	122.217	19.99	15
P5	6.0	lys	131.184	19.18	15
P1	0.0	meth	23.773	6.99	15
P1	0.5	meth	26.807	3.91	15
P1	1.0	meth	26.063	3.15	15
P1	1.5	meth	25.107	3.41	15

protocol	time	analyte	mean	sd	n_obs
P1	2.0	meth	22.431	3.08	14
P1	24.0	meth	29.890	5.88	15
P1	3.0	meth	20.362	3.14	15
P1	6.0	meth	21.497	3.21	15
P2	0.0	meth	27.869	2.77	15
P2	0.5	meth	31.156	4.40	14
P2	1.0	meth	34.325	4.20	15
P2	1.5	meth	31.870	3.72	15
P2	2.0	meth	27.918	3.75	14
P2	24.0	meth	28.413	2.30	15
P2	3.0	meth	21.372	2.99	15
P2	6.0	meth	21.678	2.62	14
P3	0.0	meth	26.659	3.29	14
P3	0.5	meth	29.364	3.82	13
P3	1.0	meth	32.672	4.64	13
P3	1.5	meth	30.766	3.81	14
P3	2.0	meth	27.744	3.77	12
P3	24.0	meth	30.069	2.55	14
P3	3.0	meth	20.729	3.52	13
P3	6.0	meth	20.293	2.88	12
P4	0.0	meth	26.263	2.87	15
P4	0.5	meth	29.181	3.53	14
P4	1.0	meth	29.733	3.06	15
P4	1.5	meth	27.303	3.52	15
P4	2.0	meth	24.151	3.52	14
P4	24.0	meth	28.274	3.52	15
P4	3.0	meth	21.063	2.77	14
P4	6.0	meth	21.901	2.58	14
P5	0.0	meth	27.229	2.28	15
P5	0.5	meth	30.228	2.59	15
P5	1.0	meth	32.909	3.28	15
P5	1.5	meth	31.326	3.11	14
P5	2.0	meth	28.148	3.06	14
P5	24.0	meth	28.304	3.14	15

protocol	time	analyte	mean	sd	n_obs
P5	3.0	meth	20.961	2.33	15
P5	6.0	meth	20.525	3.30	15
P1	0.0	MONO_ABS	0.360	0.09	15
P1	0.5	MONO_ABS	0.350	0.08	14
P1	1.0	MONO_ABS	0.393	0.08	15
P1	1.5	MONO_ABS	0.380	0.07	15
P1	2.0	MONO_ABS	0.413	0.07	15
P1	24.0	MONO_ABS	0.367	0.09	15
P1	3.0	MONO_ABS	0.360	0.08	15
P1	6.0	MONO_ABS	0.347	0.07	15
P2	0.0	MONO_ABS	0.347	0.10	15
P2	0.5	MONO_ABS	0.679	0.20	14
P2	1.0	MONO_ABS	0.653	0.15	15
P2	1.5	MONO_ABS	0.407	0.15	15
P2	2.0	MONO_ABS	0.507	0.18	14
P2	24.0	MONO_ABS	0.347	0.11	15
P2	3.0	MONO_ABS	0.547	0.19	15
P2	6.0	MONO_ABS	0.393	0.12	14
P3	0.0	MONO_ABS	0.357	0.08	14
P3	0.5	MONO_ABS	0.646	0.14	13
P3	1.0	MONO_ABS	0.579	0.15	14
P3	1.5	MONO_ABS	0.329	0.11	14
P3	2.0	MONO_ABS	0.477	0.17	13
P3	24.0	MONO_ABS	0.357	0.10	14
P3	3.0	MONO_ABS	0.521	0.15	14
P3	6.0	MONO_ABS	0.400	0.10	11
P4	0.0	MONO_ABS	0.360	0.12	15
P4	0.5	MONO_ABS	0.564	0.22	14
P4	1.0	MONO_ABS	0.593	0.20	15
P4	1.5	MONO_ABS	0.360	0.13	15
P4	2.0	MONO_ABS	0.407	0.08	14
P4	24.0	MONO_ABS	0.350	0.08	16
P4	3.0	MONO_ABS	0.433	0.13	15
P4	6.0	MONO_ABS	0.350	0.06	14

protocol	time	analyte	mean	sd	n_obs
P5	0.0	MONO_ABS	0.333	0.08	15
P5	0.5	MONO_ABS	0.673	0.13	15
P5	1.0	MONO_ABS	0.620	0.11	15
P5	1.5	MONO_ABS	0.380	0.12	15
P5	2.0	MONO_ABS	0.487	0.17	15
P5	24.0	MONO_ABS	0.343	0.08	14
P5	3.0	MONO_ABS	0.533	0.14	15
P5	6.0	MONO_ABS	0.400	0.12	15
P1	0.0	NEUTRO_ABS	2.693	0.70	15
P1	0.5	NEUTRO_ABS	2.886	0.72	14
P1	1.0	NEUTRO_ABS	3.320	1.08	15
P1	1.5	NEUTRO_ABS	3.567	1.15	15
P1	2.0	NEUTRO_ABS	3.693	1.31	15
P1	24.0	NEUTRO_ABS	2.753	0.74	15
P1	3.0	NEUTRO_ABS	3.693	1.25	15
P1	6.0	NEUTRO_ABS	3.547	1.11	15
P2	0.0	NEUTRO_ABS	2.627	0.68	15
P2	0.5	NEUTRO_ABS	4.350	1.14	14
P2	1.0	NEUTRO_ABS	4.387	1.21	15
P2	1.5	NEUTRO_ABS	5.333	1.80	15
P2	2.0	NEUTRO_ABS	7.593	2.81	14
P2	24.0	NEUTRO_ABS	2.453	0.75	15
P2	3.0	NEUTRO_ABS	9.853	2.79	15
P2	6.0	NEUTRO_ABS	7.129	2.05	14
P3	0.0	NEUTRO_ABS	2.550	0.46	14
P3	0.5	NEUTRO_ABS	4.308	0.83	13
P3	1.0	NEUTRO_ABS	4.614	1.19	14
P3	1.5	NEUTRO_ABS	5.357	1.94	14
P3	2.0	NEUTRO_ABS	7.908	2.54	13
P3	24.0	NEUTRO_ABS	2.600	0.56	14
P3	3.0	NEUTRO_ABS	9.671	2.17	14
P3	6.0	NEUTRO_ABS	6.755	1.15	11
P4	0.0	NEUTRO_ABS	2.447	0.46	15
P4	0.5	NEUTRO_ABS	3.764	0.62	14

protocol	time	analyte	mean	sd	n_obs
P4	1.0	NEUTRO_ABS	4.180	0.77	15
P4	1.5	NEUTRO_ABS	3.480	0.72	15
P4	2.0	NEUTRO_ABS	4.279	1.05	14
P4	24.0	NEUTRO_ABS	2.394	0.71	16
P4	3.0	NEUTRO_ABS	5.167	1.16	15
P4	6.0	NEUTRO_ABS	4.250	0.88	14
P5	0.0	NEUTRO_ABS	2.313	0.44	15
P5	0.5	NEUTRO_ABS	4.033	0.75	15
P5	1.0	NEUTRO_ABS	4.233	0.88	15
P5	1.5	NEUTRO_ABS	5.187	3.33	15
P5	2.0	NEUTRO_ABS	7.840	3.86	15
P5	24.0	NEUTRO_ABS	2.579	0.67	14
P5	3.0	NEUTRO_ABS	9.953	2.99	15
P5	6.0	NEUTRO_ABS	7.380	2.19	15
P1	0.0	pheala	54.634	6.27	15
P1	0.5	pheala	61.791	9.59	15
P1	1.0	pheala	61.563	5.89	15
P1	1.5	pheala	54.876	6.16	15
P1	2.0	pheala	47.805	6.99	14
P1	24.0	pheala	56.921	7.62	15
P1	3.0	pheala	45.890	5.34	15
P1	6.0	pheala	51.043	6.45	15
P2	0.0	pheala	56.884	7.51	15
P2	0.5	pheala	65.539	7.51	14
P2	1.0	pheala	74.670	10.13	15
P2	1.5	pheala	64.827	8.49	15
P2	2.0	pheala	57.262	8.98	14
P2	24.0	pheala	55.936	7.72	15
P2	3.0	pheala	48.739	7.48	15
P2	6.0	pheala	53.924	6.39	14
P3	0.0	pheala	54.996	6.58	14
P3	0.5	pheala	62.772	6.92	13
P3	1.0	pheala	69.712	9.90	13
P3	1.5	pheala	66.536	7.99	14

protocol	time	analyte	mean	sd	n_obs
P3	2.0	pheala	60.248	8.23	12
P3	24.0	pheala	57.286	7.66	14
P3	3.0	pheala	50.373	5.73	13
P3	6.0	pheala	53.458	6.55	12
P4	0.0	pheala	55.955	6.68	15
P4	0.5	pheala	66.379	7.35	14
P4	1.0	pheala	67.224	7.54	15
P4	1.5	pheala	55.965	7.43	15
P4	2.0	pheala	48.561	6.21	14
P4	24.0	pheala	56.953	7.97	15
P4	3.0	pheala	44.199	7.09	14
P4	6.0	pheala	50.576	6.52	14
P5	0.0	pheala	55.789	8.36	15
P5	0.5	pheala	63.676	9.20	15
P5	1.0	pheala	70.226	10.35	15
P5	1.5	pheala	63.414	8.61	14
P5	2.0	pheala	53.442	5.17	14
P5	24.0	pheala	56.129	8.37	15
P5	3.0	pheala	46.735	7.22	15
P5	6.0	pheala	51.585	7.32	15
P1	0.0	POTASSIUM	4.253	0.38	15
P1	0.5	POTASSIUM	4.207	0.33	15
P1	1.0	POTASSIUM	4.300	0.28	15
P1	1.5	POTASSIUM	4.393	0.20	14
P1	2.0	POTASSIUM	4.460	0.20	15
P1	24.0	POTASSIUM	4.320	0.32	15
P1	3.0	POTASSIUM	4.380	0.22	15
P1	6.0	POTASSIUM	4.140	0.34	15
P2	0.0	POTASSIUM	4.140	0.28	15
P2	0.5	POTASSIUM	5.271	0.38	14
P2	1.0	POTASSIUM	5.313	0.32	15
P2	1.5	POTASSIUM	4.393	0.22	15
P2	2.0	POTASSIUM	4.679	0.23	14
P2	24.0	POTASSIUM	4.373	0.25	15

protocol	time	analyte	mean	sd	n_obs
P2	3.0	POTASSIUM	4.287	0.30	15
P2	6.0	POTASSIUM	4.093	0.25	14
P3	0.0	POTASSIUM	4.179	0.27	14
P3	0.5	POTASSIUM	5.292	0.31	13
P3	1.0	POTASSIUM	5.129	0.49	14
P3	1.5	POTASSIUM	4.321	0.16	14
P3	2.0	POTASSIUM	4.542	0.22	12
P3	24.0	POTASSIUM	4.264	0.21	14
P3	3.0	POTASSIUM	4.457	0.29	14
P3	6.0	POTASSIUM	4.091	0.23	11
P4	0.0	POTASSIUM	4.140	0.19	15
P4	0.5	POTASSIUM	4.936	0.19	14
P4	1.0	POTASSIUM	4.987	0.25	15
P4	1.5	POTASSIUM	4.393	0.26	15
P4	2.0	POTASSIUM	4.429	0.23	14
P4	24.0	POTASSIUM	4.456	0.26	16
P4	3.0	POTASSIUM	4.373	0.22	15
P4	6.0	POTASSIUM	4.029	0.24	14
P5	0.0	POTASSIUM	4.160	0.32	15
P5	0.5	POTASSIUM	5.193	0.29	15
P5	1.0	POTASSIUM	5.227	0.36	15
P5	1.5	POTASSIUM	4.393	0.32	14
P5	2.0	POTASSIUM	4.650	0.35	14
P5	24.0	POTASSIUM	4.293	0.30	14
P5	3.0	POTASSIUM	4.453	0.29	15
P5	6.0	POTASSIUM	4.133	0.26	15
P2	15.0	RPE	13.067	2.05	15
P2	30.0	RPE	15.929	1.98	15
P2	45.0	RPE	17.200	1.74	15
P2	60.0	RPE	17.633	2.14	15
P3	15.0	RPE	13.214	1.48	15
P3	30.0	RPE	16.643	1.98	15
P3	45.0	RPE	17.643	1.98	15
P3	60.0	RPE	18.500	1.02	15

protocol	time	analyte	mean	sd	n_obs
P4	15.0	RPE	10.400	1.80	15
P4	30.0	RPE	11.267	1.75	15
P4	45.0	RPE	11.600	1.59	15
P4	60.0	RPE	11.667	1.76	15
P5	15.0	RPE	14.267	1.83	15
P5	30.0	RPE	15.667	2.50	15
P5	45.0	RPE	16.333	2.44	15
P5	60.0	RPE	17.214	2.78	14
P1	0.0	ser	110.137	20.69	15
P1	0.5	ser	122.405	26.92	15
P1	1.0	ser	115.221	20.39	15
P1	1.5	ser	164.895	40.34	15
P1	2.0	ser	134.039	29.98	14
P1	24.0	ser	108.899	18.30	15
P1	3.0	ser	97.735	18.24	15
P1	6.0	ser	95.578	15.22	15
P2	0.0	ser	109.785	16.97	15
P2	0.5	ser	113.911	21.46	14
P2	1.0	ser	120.974	22.24	15
P2	1.5	ser	155.337	33.57	15
P2	2.0	ser	131.510	32.18	14
P2	24.0	ser	110.520	15.30	15
P2	3.0	ser	89.275	16.02	15
P2	6.0	ser	90.551	14.52	14
P3	0.0	ser	109.586	21.17	14
P3	0.5	ser	106.121	20.33	13
P3	1.0	ser	112.288	20.53	13
P3	1.5	ser	128.254	21.73	14
P3	2.0	ser	131.744	21.63	12
P3	24.0	ser	109.442	16.08	14
P3	3.0	ser	91.072	20.43	13
P3	6.0	ser	86.987	15.78	12
P4	0.0	ser	107.499	14.70	15
P4	0.5	ser	123.148	17.56	14

protocol	time	analyte	mean	sd	n_obs
P4	1.0	ser	124.685	19.28	15
P4	1.5	ser	163.015	34.77	15
P4	2.0	ser	122.660	23.10	14
P4	24.0	ser	105.415	17.82	15
P4	3.0	ser	94.545	13.81	14
P4	6.0	ser	91.038	13.16	14
P5	0.0	ser	108.352	17.43	15
P5	0.5	ser	112.443	19.50	15
P5	1.0	ser	117.523	21.31	15
P5	1.5	ser	155.390	34.51	14
P5	2.0	ser	137.091	28.69	14
P5	24.0	ser	106.727	17.29	15
P5	3.0	ser	87.053	15.78	15
P5	6.0	ser	88.150	16.86	15
P1	0.0	SODIUM	139.200	4.31	15
P1	0.5	SODIUM	138.867	3.91	15
P1	1.0	SODIUM	138.667	4.17	15
P1	1.5	SODIUM	138.214	3.56	14
P1	2.0	SODIUM	138.067	3.54	15
P1	24.0	SODIUM	138.200	2.48	15
P1	3.0	SODIUM	137.800	3.76	15
P1	6.0	SODIUM	138.333	3.29	15
P2	0.0	SODIUM	138.400	2.56	15
P2	0.5	SODIUM	139.643	2.17	14
P2	1.0	SODIUM	139.333	3.15	15
P2	1.5	SODIUM	136.733	2.19	15
P2	2.0	SODIUM	136.500	2.56	14
P2	24.0	SODIUM	138.933	2.71	15
P2	3.0	SODIUM	135.133	2.50	15
P2	6.0	SODIUM	136.071	2.34	14
P3	0.0	SODIUM	138.571	2.90	14
P3	0.5	SODIUM	140.692	2.50	13
P3	1.0	SODIUM	141.286	2.09	14
P3	1.5	SODIUM	140.429	2.17	14

protocol	time	analyte	mean	sd	n_obs
P3	2.0	SODIUM	139.417	2.47	12
P3	24.0	SODIUM	138.000	2.72	14
P3	3.0	SODIUM	138.571	2.53	14
P3	6.0	SODIUM	135.909	2.43	11
P4	0.0	SODIUM	138.400	2.20	15
P4	0.5	SODIUM	139.786	2.78	14
P4	1.0	SODIUM	137.933	2.94	15
P4	1.5	SODIUM	137.267	3.04	15
P4	2.0	SODIUM	136.429	2.56	14
P4	24.0	SODIUM	139.062	2.82	16
P4	3.0	SODIUM	136.400	2.13	15
P4	6.0	SODIUM	136.643	2.79	14
P5	0.0	SODIUM	139.067	2.34	15
P5	0.5	SODIUM	141.067	1.91	15
P5	1.0	SODIUM	140.867	2.56	15
P5	1.5	SODIUM	137.214	2.49	14
P5	2.0	SODIUM	136.214	1.63	14
P5	24.0	SODIUM	139.214	2.99	14
P5	3.0	SODIUM	136.533	2.33	15
P5	6.0	SODIUM	136.867	2.62	15
P1	0.0	tau	108.993	44.16	15
P1	0.5	tau	100.521	36.98	15
P1	1.0	tau	96.034	36.80	15
P1	1.5	tau	116.768	37.86	15
P1	2.0	tau	106.074	31.90	14
P1	24.0	tau	107.864	49.35	15
P1	3.0	tau	101.555	30.97	15
P1	6.0	tau	101.153	36.37	15
P2	0.0	tau	131.534	32.56	15
P2	0.5	tau	205.882	49.88	14
P2	1.0	tau	218.251	55.94	15
P2	1.5	tau	175.149	45.43	15
P2	2.0	tau	174.017	45.91	14
P2	24.0	tau	119.865	30.87	15

protocol	time	analyte	mean	sd	n_obs
P2	3.0	tau	140.087	41.51	15
P2	6.0	tau	134.881	44.35	14
P3	0.0	tau	114.461	27.01	14
P3	0.5	tau	164.887	50.96	13
P3	1.0	tau	175.897	50.31	13
P3	1.5	tau	143.324	29.98	14
P3	2.0	tau	141.351	31.45	12
P3	24.0	tau	117.095	28.02	14
P3	3.0	tau	134.805	26.72	13
P3	6.0	tau	119.589	34.76	12
P4	0.0	tau	130.336	32.23	15
P4	0.5	tau	147.686	34.05	14
P4	1.0	tau	172.787	41.64	15
P4	1.5	tau	160.081	40.17	15
P4	2.0	tau	150.638	30.13	14
P4	24.0	tau	129.142	32.13	15
P4	3.0	tau	135.021	31.60	14
P4	6.0	tau	124.516	25.77	14
P5	0.0	tau	119.477	26.11	15
P5	0.5	tau	191.724	58.37	15
P5	1.0	tau	192.202	45.19	15
P5	1.5	tau	161.129	33.28	14
P5	2.0	tau	163.966	32.56	14
P5	24.0	tau	113.212	20.27	15
P5	3.0	tau	136.903	38.72	15
P5	6.0	tau	128.459	35.93	15
P1	0.0	thre	113.530	19.85	15
P1	0.5	thre	123.382	23.00	15
P1	1.0	thre	121.530	21.91	15
P1	1.5	thre	175.467	43.35	15
P1	2.0	thre	142.070	27.12	14
P1	24.0	thre	125.839	24.52	15
P1	3.0	thre	104.407	22.46	15
P1	6.0	thre	98.705	15.76	15

protocol	time	analyte	mean	sd	n_obs
P2	0.0	thre	118.883	23.42	15
P2	0.5	thre	122.514	27.81	14
P2	1.0	thre	132.140	27.36	15
P2	1.5	thre	175.124	45.21	15
P2	2.0	thre	151.286	39.78	14
P2	24.0	thre	115.111	18.40	15
P2	3.0	thre	100.965	25.65	15
P2	6.0	thre	96.794	20.76	14
P3	0.0	thre	117.287	23.45	14
P3	0.5	thre	115.460	22.82	13
P3	1.0	thre	124.366	24.51	13
P3	1.5	thre	140.184	32.15	14
P3	2.0	thre	149.125	36.13	12
P3	24.0	thre	118.903	21.74	14
P3	3.0	thre	102.716	23.36	13
P3	6.0	thre	92.430	19.12	12
P4	0.0	thre	117.127	23.21	15
P4	0.5	thre	129.319	28.75	14
P4	1.0	thre	131.131	25.81	15
P4	1.5	thre	179.913	48.90	15
P4	2.0	thre	138.234	32.84	14
P4	24.0	thre	111.173	19.29	15
P4	3.0	thre	103.200	23.57	14
P4	6.0	thre	95.536	17.49	14
P5	0.0	thre	117.605	19.92	15
P5	0.5	thre	122.532	23.77	15
P5	1.0	thre	129.174	24.87	15
P5	1.5	thre	171.699	38.86	14
P5	2.0	thre	159.105	30.11	14
P5	24.0	thre	112.059	18.11	15
P5	3.0	thre	100.059	17.93	15
P5	6.0	thre	95.220	17.22	15
P1	0.0	trp	58.541	7.40	15
P1	0.5	trp	62.671	8.52	15

protocol	time	analyte	mean	sd	n_obs
P1	1.0	trp	60.946	6.91	15
P1	1.5	trp	57.989	6.89	15
P1	2.0	trp	53.432	5.10	14
P1	24.0	trp	59.871	8.83	15
P1	3.0	trp	51.865	5.07	15
P1	6.0	trp	52.782	5.85	15
P2	0.0	trp	64.367	9.72	15
P2	0.5	trp	70.021	9.38	14
P2	1.0	trp	77.427	10.50	15
P2	1.5	trp	71.329	8.94	15
P2	2.0	trp	66.874	9.08	14
P2	24.0	trp	55.389	6.26	15
P2	3.0	trp	52.788	7.85	15
P2	6.0	trp	53.530	5.39	14
P3	0.0	trp	59.710	7.31	14
P3	0.5	trp	63.926	8.47	13
P3	1.0	trp	68.867	7.36	13
P3	1.5	trp	67.081	7.63	14
P3	2.0	trp	65.838	7.28	12
P3	24.0	trp	55.545	7.32	14
P3	3.0	trp	53.574	4.96	13
P3	6.0	trp	51.325	7.15	12
P4	0.0	trp	62.611	7.83	15
P4	0.5	trp	69.582	7.57	14
P4	1.0	trp	72.244	7.34	15
P4	1.5	trp	64.756	8.70	15
P4	2.0	trp	61.096	7.71	14
P4	24.0	trp	61.311	7.23	15
P4	3.0	trp	55.459	7.72	14
P4	6.0	trp	56.159	7.44	14
P5	0.0	trp	61.812	6.73	15
P5	0.5	trp	67.944	9.26	15
P5	1.0	trp	70.791	5.91	15
P5	1.5	trp	67.312	6.67	14

protocol	time	analyte	mean	sd	n_obs
P5	2.0	trp	63.705	9.79	14
P5	24.0	trp	53.569	5.23	15
P5	3.0	trp	52.593	7.99	15
P5	6.0	trp	53.091	5.70	15
P1	0.0	tyr	59.207	7.50	15
P1	0.5	tyr	65.100	12.13	15
P1	1.0	tyr	64.245	8.54	15
P1	1.5	tyr	60.319	8.36	15
P1	2.0	tyr	54.645	6.01	14
P1	24.0	tyr	63.545	9.98	15
P1	3.0	tyr	49.233	7.45	15
P1	6.0	tyr	46.047	6.23	15
P2	0.0	tyr	60.167	9.17	15
P2	0.5	tyr	69.839	10.99	14
P2	1.0	tyr	81.021	13.40	15
P2	1.5	tyr	80.145	14.58	15
P2	2.0	tyr	75.100	15.12	14
P2	24.0	tyr	55.801	6.74	15
P2	3.0	tyr	60.360	12.50	15
P2	6.0	tyr	50.687	6.74	14
P3	0.0	tyr	57.621	8.28	14
P3	0.5	tyr	64.153	8.98	13
P3	1.0	tyr	71.300	10.51	13
P3	1.5	tyr	75.137	13.22	14
P3	2.0	tyr	73.032	13.95	12
P3	24.0	tyr	57.464	7.63	14
P3	3.0	tyr	59.232	8.57	13
P3	6.0	tyr	48.025	6.57	12
P4	0.0	tyr	59.997	8.85	15
P4	0.5	tyr	73.626	12.65	14
P4	1.0	tyr	78.548	11.36	15
P4	1.5	tyr	70.491	10.83	15
P4	2.0	tyr	64.346	10.32	14
P4	24.0	tyr	57.999	7.53	15

protocol	time	analyte	mean	sd	n_obs
P4	3.0	tyr	53.842	8.65	14
P4	6.0	tyr	47.897	5.76	14
P5	0.0	tyr	60.246	7.47	15
P5	0.5	tyr	69.611	8.09	15
P5	1.0	tyr	79.681	9.69	15
P5	1.5	tyr	79.097	9.07	14
P5	2.0	tyr	73.784	10.92	14
P5	24.0	tyr	54.869	7.09	15
P5	3.0	tyr	59.800	8.98	15
P5	6.0	tyr	48.645	5.84	15
P1	0.0	UREUM	4.520	1.02	15
P1	0.5	UREUM	4.533	1.04	15
P1	1.0	UREUM	4.540	1.03	15
P1	1.5	UREUM	4.807	1.08	14
P1	2.0	UREUM	5.233	1.01	15
P1	24.0	UREUM	5.113	1.16	15
P1	3.0	UREUM	5.313	0.97	15
P1	6.0	UREUM	4.860	0.91	15
P2	0.0	UREUM	4.100	0.79	15
P2	0.5	UREUM	4.379	0.69	14
P2	1.0	UREUM	4.480	0.78	15
P2	1.5	UREUM	5.260	0.84	15
P2	2.0	UREUM	5.779	0.64	14
P2	24.0	UREUM	5.233	0.85	15
P2	3.0	UREUM	5.920	0.81	15
P2	6.0	UREUM	5.264	0.73	14
P3	0.0	UREUM	4.921	1.01	14
P3	0.5	UREUM	5.261	0.92	13
P3	1.0	UREUM	5.300	1.04	14
P3	1.5	UREUM	5.529	1.06	14
P3	2.0	UREUM	6.233	0.95	12
P3	24.0	UREUM	5.414	1.04	14
P3	3.0	UREUM	6.879	0.94	14
P3	6.0	UREUM	6.409	0.90	11

protocol	time	analyte	mean	sd	n_obs
P4	0.0	UREUM	4.307	0.83	15
P4	0.5	UREUM	4.464	0.78	14
P4	1.0	UREUM	4.527	0.86	15
P4	1.5	UREUM	4.987	0.87	15
P4	2.0	UREUM	5.557	0.78	14
P4	24.0	UREUM	5.013	0.86	16
P4	3.0	UREUM	5.547	0.80	15
P4	6.0	UREUM	4.993	0.75	14
P5	0.0	UREUM	4.500	1.04	15
P5	0.5	UREUM	4.720	1.07	15
P5	1.0	UREUM	4.947	1.10	15
P5	1.5	UREUM	5.364	1.11	14
P5	2.0	UREUM	5.921	1.08	14
P5	24.0	UREUM	5.457	0.72	14
P5	3.0	UREUM	6.180	1.14	15
P5	6.0	UREUM	5.633	1.37	15
P1	0.0	val	210.186	19.00	15
P1	0.5	val	224.095	25.08	15
P1	1.0	val	219.326	20.17	15
P1	1.5	val	210.981	19.75	15
P1	2.0	val	196.701	23.32	14
P1	24.0	val	240.763	20.76	15
P1	3.0	val	188.041	19.26	15
P1	6.0	val	201.363	21.30	15
P2	0.0	val	215.245	17.65	15
P2	0.5	val	227.773	19.61	14
P2	1.0	val	237.535	22.43	15
P2	1.5	val	221.634	18.18	15
P2	2.0	val	205.915	20.08	14
P2	24.0	val	233.320	26.04	15
P2	3.0	val	186.058	19.30	15
P2	6.0	val	209.459	22.49	14
P3	0.0	val	216.467	28.97	14
P3	0.5	val	223.375	28.02	13

protocol	time	analyte	mean	sd	n_obs
P3	1.0	val	228.102	29.92	13
P3	1.5	val	227.349	29.12	14
P3	2.0	val	217.208	31.01	12
P3	24.0	val	224.069	23.59	14
P3	3.0	val	187.768	24.90	13
P3	6.0	val	200.863	20.71	12
P4	0.0	val	216.990	25.30	15
P4	0.5	val	239.795	24.66	14
P4	1.0	val	237.510	25.51	15
P4	1.5	val	217.047	23.91	15
P4	2.0	val	200.819	21.38	14
P4	24.0	val	231.987	29.18	15
P4	3.0	val	183.581	21.51	14
P4	6.0	val	201.750	20.84	14
P5	0.0	val	220.193	28.26	15
P5	0.5	val	229.465	29.69	15
P5	1.0	val	234.389	27.42	15
P5	1.5	val	224.003	32.07	14
P5	2.0	val	205.066	30.13	14
P5	24.0	val	238.481	28.79	15
P5	3.0	val	193.058	37.89	15
P5	6.0	val	219.103	48.74	15
P1	0.0	Zonulin	28.253	8.38	12
P1	1.0	Zonulin	31.286	8.80	12
P1	1.5	Zonulin	30.300	NA	1
P1	2.0	Zonulin	31.499	4.87	11
P1	24.0	Zonulin	31.820	6.25	12
P2	0.0	Zonulin	30.566	7.57	13
P2	1.0	Zonulin	34.672	4.82	13
P2	2.0	Zonulin	31.844	3.12	13
P2	24.0	Zonulin	33.849	4.44	13
P3	0.0	Zonulin	36.720	6.60	12
P3	0.5	Zonulin	33.210	NA	1
P3	1.0	Zonulin	37.092	6.01	12

protocol	time	analyte	mean	sd	n_obs
P3	1.5	Zonulin	42.890	0.35	2
P3	2.0	Zonulin	39.990	4.86	11
P3	24.0	Zonulin	36.287	9.61	12
P3	3.0	Zonulin	43.780	NA	1
P3	6.0	Zonulin	34.600	NA	1
P4	0.0	Zonulin	31.454	7.98	12
P4	1.0	Zonulin	33.087	7.37	12
P4	2.0	Zonulin	31.714	7.06	12
P4	24.0	Zonulin	30.561	5.86	12