Manual

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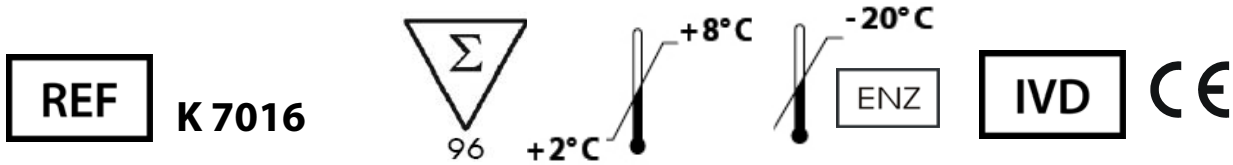
BCAA Kit

For the in vitro determination of L-leucine, L-isoleucine and L-valine in EDTA plasma and serum

Gültig ab / Valid from 2016-07-06



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1. INTENDED USE

This enzyme test is intended for the determination of branched-chain amino acids (L-leucine, L-isoleucine, L-valine) in EDTA plasma and serum. It is for *in vitro* diagnostic use only.

2. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
K 7016	PLATE	Microtiter plate	12 x 8 wells
K 7016	STD	Standards, lyophilized (0, 100, 300, 1000 µmol/l)	4 x 1 vial
K 7016	CTRL 1	Control, lyophilized (see specification for range)	2 x 1 vial
K 7016	CTRL 2	Control, lyophilized (see specification for range)	2 x 1 vial
K 7016	ASYBUF	Assay buffer, ready to use	16 ml
K 7016	REABUF	Reaction buffer, lyophilized	2 x 1 vial
K 7016	ENZ	Leucine dehydrogenase, concentrate	2 x 1 vial

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

3. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Calibrated precision pipets and 10-1000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Centrifuge, 3000 x *g*
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 340 nm

* Immundiagnostik AG recommends the use of Ultra Pure 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 (≥18.2 MΩ cm).

4. 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 assay**. 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.
- Reconstitute the lyophilized **Standards (STD) and controls (CTRL1, CTRL2)** with **2.5 ml** of **ultra pure water,** mix well. The dissolved standards and controls can be stored at **-20°C**. Re-freeze immediately after use.
- Dissolve the content of one vial of **reaction buffer (REABUF)** in **3.0 ml** of **ultra pure water,** mix well. When more than one vial is to be used, combine the contents and mix prior to use. The dissolved reaction buffer can be stored at **-20°C** for **6 months**.
- The enzyme **leucine dehydrogenase (ENZ)** is stored at -**20 °C**. Before use add **2.4 ml** of **assay buffer (ASYBUF)** to the content of one vial of ENZ and mix well. When more than one vial is to be used, combine the contents and mix prior to use. The diluted ENZ can be stored at **-20°C** for **6 months**.
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2-8 °C.**

5. PREPARATION AND STORAGE OF SAMPLES

EDTA plasma and serum

- Samples are stable for 48 h at 2-8°C. For longer storage keep samples frozen at -20°C.
- Samples are analyzed **undiluted**.

6. ASSAY PROCEDURE

Principle of the test

This assay is a photometric test intended for the determination of leucine, isoleucine and valine by enzymatic dehydration, in which NAD^+ is transformed to NADH.

In this reaction the amino acids L-leucine, L-isoleucine, L-valine are oxidized to the respective α-ketone bodies by reducing NAD^+ to NADH. This reaction can be measured at 340 nm and it is proportional to the amount of oxidized BCAA.

Test procedure

Bring all **reagents and samples 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 (PLATE) as needed from the kit. Store unused strips covered at 2-8 °C. Strips are stable until the expiry date stated on the label.

We recommend to carry out the tests in duplicate.

1.	Add **2 x 25 µl** of **standards/ controls/ samples (STD/ CTRL/ SAMPLE)** into the respective wells of the microtiter plate (PLATE).
2.	Add **100 µl** of **ultra pure water** into each well.
3.	Add **50 µl** of **assay buffer (ASYBUF)** into each well.
4.	Add **50 µl** of **reaction buffer (READBUF)** into each well and determine absorption **immediately** with an ELISA reader at **340 nm (OD $_{BLANK}$)**.
5.	Add **50 µl** of diluted **leucine dehydrogenase (ENZ)** into each well. Cover plate tightly.
6.	Incubate for **60 minutes** at **room temperature**.
7.	Determine absorption at **340 nm (OD $_{SAMPLE}$)**.
8.	For analysis of obtained data see chapter 9 “results”.

7. RESULTS

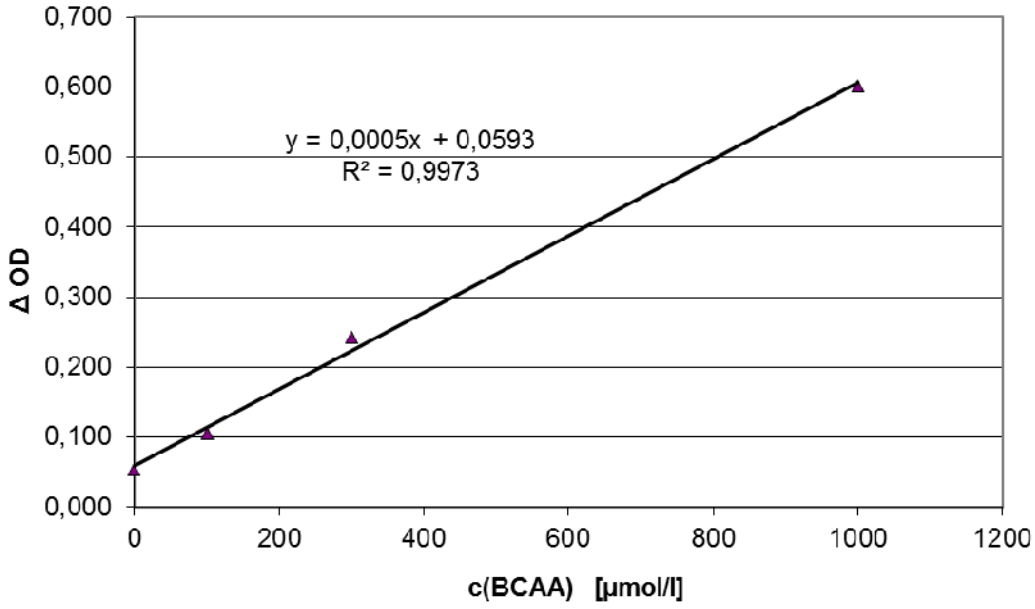
For calculation of results subtract OD values of the blank (OD_{BLANK}) from OD values after the addition of enzyme (OD_{SAMPLE}):

$$\Delta OD = OD_{SAMPLE} - OD_{BLANK}$$

To generate a standard curve, Δ OD of the standards are plotted against the standard concentrations. With the obtained slope and y-intercept BCAA concentrations of the samples can be calculated:

$$\text{BCAA [µmol/l]} = \frac{\Delta OD - \text{intercept}}{\text{slope}}$$

In the following, an example of a calibration curve is given; do not use it for the calculation of your results.



8. LIMITATIONS

Samples with an OD higher than the OD of the highest standard should be diluted with ultra pure water and re-assayed. Please consider this dilution factor when calculating the results.

9. QUALITY CONTROL

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

Control samples should be analyzed 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 samples are outside of the acceptable limits.

Reference Range

Based on internal studies with plasma samples of evidently healthy persons (n = 146) a mean value of 460 µmol/l was calculated. The standard deviation was 110.5 µmol/l.

Plasma mean value ± 2 x standard deviation: **460 ± 221 µmol/l**

Normal range: **239 – 681 µmol/l**

We recommend each laboratory to establish its own reference range.

10. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Serum

Intra assay (n = 6)

sample	BCAA [µmol/l]	CV [%]
1	448	5.5
2	382	4.2

Inter assay (n = 6)

sample	BCAA [µmol/l]	CV [%]
1	465	3.7
2	193	8.9
3	425	6.6
4	171	8.0

EDTA plasma

Intra assay (n = 6)

sample	BCAA [µmol/l]	CV [%]
1	453	9.3
2	1337	2.6

Inter assay (n = 6)

sample	BCAA [µmol/l]	CV [%]
1	1627	3.1
2	828	3.6
3	1418	4.1
4	706	4.0

Spiking recovery

Two plasma and serum samples were spiked with different BCAA concentrations and measured in this assay. The mean recovery rate was 93.8 % for EDTA plasma and 94.3 % for serum (n = 2).

EDTA plasma

sample	spike [µmol/l]	BCAA expected [µmol/l]	BCAA measured [µmol/l]	recovery [%]
A			577	
	1500	1039	952	91.7
	3000	1789	1665	93.1
B			441	
	1500	970	895	92.2
	3000	1720	1691	98.2

Serum

sample	spike [µmol/l]	BCAA expected [µmol/l]	BCAA measured [µmol/l]	recovery [%]
A			387	
	1500	944	938	99.4
	3000	1694	1551	91.6
B			453	
	1500	977	901	92.2
	3000	1727	1625	94.1

Dilution recovery

Two serum and plasma samples were diluted with ultra pure water and measured in this assay. The mean recovery rate was 95,4 % for EDTA plasma and 88.1 % for serum (n = 2).

EDTA plasma

sample	dilution	BCAA expected [µmol/l]	BCAA measured [µmol/l]	recovery [%]
A			577	
	1:2	289	266	92.1
B			1590	
	1:2	795	785	100.0

Serum

sample	dilution	BCAA expected [µmol/l]	BCAA measured [µmol/l]	recovery [%]
A			167	
	1:2	194	266	87.6
B			201	
	1:2	227	785	88.6

Analytical sensitivity

STD 1 (zero-standard) was measured 14 times. The detection limit was set as B_0 + 2 SD and estimated to be 5.8 µmol/l.

Sample	mean value [OD]	2 x standard deviation (SD)	Detection limit [µmol/l]
STD 1	0.06	0.0023	5.8

Specificity

Specificity was tested by measuring the cross-reactivity against compounds with structural similarity to kynurenic acid. No cross reactivity with L-methionin was found.

11. 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 color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes
- The stop solution consists of sulfuric acid, which is a strong acid. Although diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped up immediately with copious quantities of water. Do not breathe vapour and avoid inhalation.

12. 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 analyzed with each run.
- Reagents should not be used beyond the expiration date stated on the 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.

13. 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.
- Incubation time, incubation temperature, and pipetting volumes of the different 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 sent to Immundiagnostik AG along with a written complaint.

14. REFERENCES

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Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention

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