Manual

Glutamine Kit

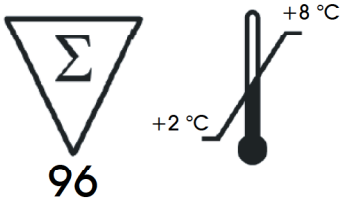
For the determination of glutamine in human EDTA plasma and serum

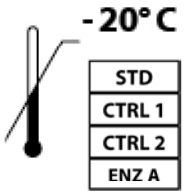
For research use only

Valid from 09.06.2010

REF

K 7732





RUO

1. INTENDED USE

This enzyme test is intended for the determination of glutamine in human EDTA plasma and serum. It is for research use only.

2. INTRODUCTION

Glutamine is the most abundant free amino acid, with a concentration of about 500-900 µmol/l in human blood. The most relevant glutamine-producing tissue is the muscle mass, accounting for about 90% of all glutamine synthesized. The most eager consumers of glutamine are the cells of intestines, the kidney cells for the acid base balance, activated immune cells and many cancer cells.

In catabolic states of injury and illness, glutamine becomes conditionally-essential (requiring intake from food or supplements). It is also known that glutamine has various effects in reducing healing time after operations. Clinical trials have revealed that providing parenteral nutrition regimes containing high amounts of glutamine to patients improve nitrogen balance, maintain the intracellular Gln pool, preserve intestinal permeability and absorption, and reduce hospital-stay times.

Glutamine is also marketed as a supplement used for muscle growth in weightlifting, bodybuilding, endurance, and other sports, yet there is still no scientific evidence of its "muscle-growing" properties. In biological research, L-glutamine is commonly added to the media in cell culture.

Glutamine is closely related to the excitatory amino acid glutamate. By the glutamate-glutamine cycle adequate supply of the neurotransmitter glutamate is maintained in the central nervous system: Glial cells release glutamine, which is then taken up into presynaptic terminals, metabolized into glutamate, and packaged into synaptic vesicles by the glutamate transporter VGLUT. Once the vesicle is released, glutamate is removed from the synaptic cleft by excitatory amino acid transporters (EAAT) into glial cells, where it is then converted into glutamine and transported out of the cells into the nerve terminal.

At GABAergic synapses, the cycle is called the GABA-glutamine cycle. Here the glutamine taken up by neurons is converted to glutamate, which is then metabolized into GABA. Upon release, GABA is taken up by glial cells via GABA transporters, metabolized in a series of steps back to glutamine via glutamate.

3. PRINCIPLE OF THE TEST

This assay is a photometric test intended for the determination of L-glutamine. In this test L-glutamine is enzymatically deaminated to L-glutamate, which subsequently is dehydrated by transforming NAD^+ to NADH.

The measurement of L-glutamine is a two-step reaction:

A) Deamination of L-glutamine to L-glutamate, and

B) Dehydration of L-glutamate to α-ketoglutarate by reducing NAD^+ to NADH. This reaction can be measured at 340 nm. It is proportional to the amount of oxidized L-glutamate, and therefore also to the amount of L-glutamine which has been transformed to glutamate.

In this way the assay measures total glutamine/glutamate content of the sample (step A and B). Measuring subsequently the natural glutamtate content of the sample (only step B) allows calculation of original glutamine content of the sample

D-isomers are not detected by this assay.

4. MATERIAL SUPPLIED

Catalog No	Content	Kit Components	Quantity
K7732MTP	PLATE	Microtiter plate	2 x 12 x 8 wells
K7732ST	STD	Standards	2 x 1 vial
K7732KO	CTRL 1 CTRL 2	Controls	2 x 1 vial
K7732SP	SAMPLEBUF	Sample buffer	6 ml
K7732EA	ENZ A	Glutaminase	2 x 1 vial
K7732AP	ASYBUF	Assay buffer	16 ml
K7732RP	REABUF	Reaction buffer	2 x 1 vial
K7732EB	ENZ B	Glutamate dehydrogenase	2 x 1 vial

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Double distilled water (bidest. water)
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 10000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 340 nm
- Incubator 37°C

6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 2 times within the expiry date stated on the label.
- **Standards (STD) and controls (CTRL1, CTRL2)** are lyophilized and must be reconstituted in **300 µl bidest. water**. After use standards and controls can be stored at -20°C and can be re-frozen up to 2 times. Re-freeze immediately after use.
- The content of one vial of **reaction buffer (REABUF)** must be dissolved in **3 ml bidest. water**, mix well. Discard any remaining quantity after use. By providing two REABUF vials the kit can be separated into two performances.
- The content of one vial of **glutaminase (ENZ A)** must be dissolved in **225 µl reaction buffer (SAMPLEBUF) and 1.3 ml bidest. water**, mix well. Discard any remaining quantity after use. By providing two ENZ A vials the kit can be separated into two performances.
- To the content of one vial of **glutamate dehydrogenase (ENZ B) 2.6 ml assay buffer (ASYBUF)** must be added, mix well. Discard any remaining quantity after use. By providing two ENZ B vials the kit can be separated into two performances.
- All other test reagents are stable until date of expiry (see label) when stored at 2-8°C.

7. PRECAUTIONS

- For research use only.
- Reagents should not be used beyond the expiry date shown on kit label.

8. SPECIMEN COLLECTION AND PREPARATION

EDTA plasma and serum

- Venous fasting blood is suited for this test system. As stability of fresh blood samples is very limited, they should be stored at 2-8°C and measured within 48 h. For longer storage samples should be stored frozen at -20°C.
- Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.
- If less than 50 µl of sample is provided we recommend diluting the sample 1:2 in SAMPLEBUF (25 µl sample + 25 µl SAMPLEBUF). This dilution factor must be considered in data evaluation.
- **Samples** with visible amounts of **precipitates** should be **centrifuged** at least for 5 min at 10000 x g. The resulting supernatant is used in the assay.

9. ASSAY PROCEDURE

Procedural notes

- Quality control guidelines should be observed.
- Incubation time, incubation temperature, and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure that are not coordinated with the producer may influence the test results. Immundiagnostik AG can therefore not be held reliable for any damage resulting from this.
- The assay should always be performed according to the enclosed manual.

Test procedure

A) Total concentration of Glutamine and Glutamate

1.	Mark the positions of standards (STD)/ controls (CTRL)/ samples (SAMPLE) in duplicate on a protocol sheet
2.	Take as many microtiter strips (PLATE) as needed from kit. Store unused strips covered at room temperature.
3.	Add **2 x 50 µl of standards (STD)/ controls (CTRL)/ samples (SAMPLE)** into the respective well of the microtiter plate (PLATE)
4.	Add **50 µl of assay buffer (ASYBUF)** into each well.
5.	Add **25 µl of reconstituted glutamines (ENZ A)** into each well. Cover the plate tightly
6.	Incubate at **37°C** for **1 hour**.
7.	Add **100 µl assay buffer (ASYBUF)** into each well
8.	Add **50 µl reaction buffer (REABUF)** into each well, and determine absorption **immediately** with an ELISA reader at **340 nm** (OD_{BLANK}).
9.	Add **50 µl diluted glutamate dehydrogenase (ENZ B)** into each well. Cover plate tightly.
10.	Incubate at **37°C** for **15 minutes.**
11.	Determine absorption at **340 nm** (OD_{SAMPLE}).
12.	For analysis of obtained data see chapter 10 “evaluation of results”.

B) Determination of Glutamate

13.	Mark the positions of standards (STD)/controls (CTRL)/ samples (SAMPLE) in duplicate on a protocol sheet
14.	Take as many microtiter strips (PLATE) as needed from kit. Store unused strips covered at room temperature.
15.	Add **2 x 50 µl of standards (STD) / controls (CTRL) / samples (SAMPLE)** into the respective well of the microtiter plate (PLATE)
16.	Add **50 µl of bidest. water** into each well.
17.	Add **100 µl assay buffer (ASYBUF)** into each well
18.	Add **50 µl reaction buffer (REABUF)** into each well, and determine absorption **immediately** with an ELISA reader at **340 nm** (OD_{BLANK}).
19.	Add **50 µl diluted glutamate dehydrogenase (ENZ B)** into each well. Cover plate tightly.
20.	Incubate at **37°C** for **15 minutes.**
21.	Determine absorption at **340 nm** (OD_{SAMPLE}).
22.	For analysis of obtained data see chapter 10 “evaluation of results”.

10. Evaluation of Results

If the test is performed in strict compliance with the manufacturer's instructions (i.e. with the exact volumes for standards, controls, and samples, and with correct sample treatment), standards, controls, and samples are equally diluted. Therefore, **no dilution factor is required for calculation of the results.** **

**If samples are diluted 1:2, the results must be multiplied by 2.

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

To generate a standard curve ΔOD of the standards are plotted against the standard concentrations (see quality control protocol). With the obtained slope and y-intercept glutamate concentrations of the samples can be calculated:

$$\text{glutamate concentration} = \frac{(OD_{Sample} - OD_{Blank}) - \text{intercept}}{\text{slope}} \text{ [µmol/l]}$$

Subsequently, from the total glutamine/glutamate concentration of the sample (see test procedure A), the glutamate concentration of the sample (see test procedure B) is subtracted, which gives the original concentration of glutamine in the sample.

Expected values

Based on internal studies with serum samples of evidently healthy persons **(n=24)** a mean value of 510 µmol/l was estimated. The standard variation was 82 µmol/l.

Normal range: mean value ± 2 x standard variation: 510 ± 164 µmol/l

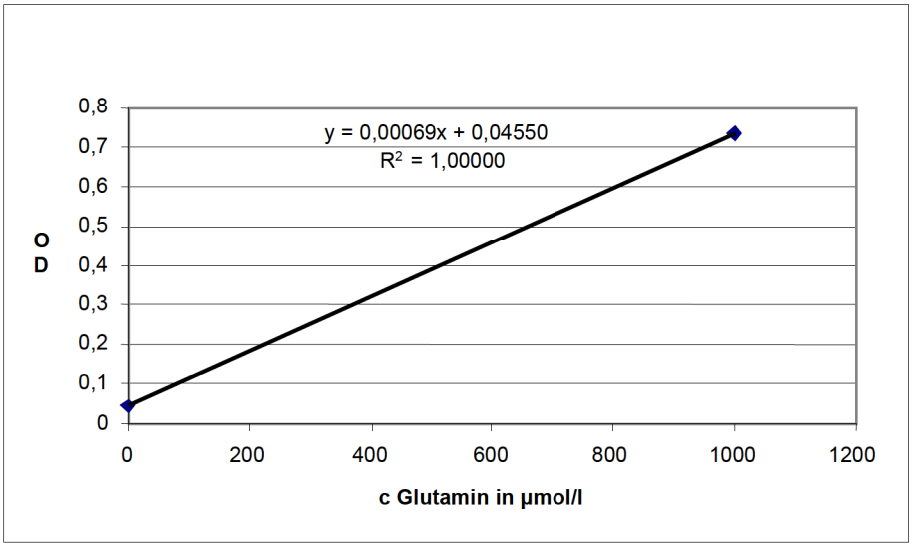
We recommend each laboratory to develop its own normal range. The values mentioned above are only for orientation and can deviate from other published data.

Controls

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 the acceptable limits.

The concentration of controls and patient samples can be determined directly from the calibration curve. In the following an example of a calibration curve is given.

Example of calibration curve



11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n=12)		
Sample	Glutamine [µmol/l]	Standard variation (SD) [%]
1	505,3	1,7
2	736,0	1,0

Inter-Assay (n=6)		
Sample	Glutamine [µmol/l]	Standard variation (SD) [%]
1	192,9	5,4
2	516,6	4,2

Sensitivity

The sensitivity was set as B_0 + 2SD. The zero-standard was measured 6 times.

Sample	Glutamine mean value [OD]	2 x Standard variation (2 x SD) [%]	Detection limit [µmol/l]
0	0,05	18,3	18,6

Recovery

One sample was spiked with different glutamine concentrations and measured in this assay. The analytical recovery rate was determined by the expected and measured glutamine levels. The expected levels were calculated as the sum of the measured glutamine concentration in the original sample and the spiked glutamine amount. The mean recovery rate for all concentrations was 100,8 % (n=6).

Spike [µmol/l]	Glutamine expected [µmol/l]	Glutamine measured [µmol/l]	Recovery [%]
0	x	x=346	100,0
150	346+150=496	493	99,4
250	346+250=596	614	103

Linearity

Linearity of the test was determined by diluting a spiked patient sample. The mean linearity was 104,6 %.

Dilution	Measured [µmol/l]	Expected [µmol/l]	Recovery [%]
original	346	346	100,0
1+1	183	173	105,8
1+3	94	87	108,0

12. LIMITATIONS

Hemolytic and lipemic samples may give erroneous results. Do not measure hemolytic and lipemic samples.

13. REFERENCES

Coëffier M, Déchelotte P: Combined infusion of glutamine and arginine: does it make sense? Curr Opin Clin Nutr Metab Care. 2010 Jan; 13(1): 70-4

Agostini F, Biolo G: Effect of physical activity on glutamine metabolism. Curr Opin Clin Nutr Metab Care. 2010 Jan; 13(1): 58-64

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for research use only.
- Reagents should not be used after the date of expiry stated on the label.
- Single components with different lot numbers should not be mixed or exchanged.
- The guidelines for medical laboratories should be observed.

- 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 wrong use.

Used symbols:

	Temperature limitation	REF	Catalogue Number
RUO	For research use only	Σ	Contains sufficient for <n> tests
	Manufacturer		Use by
LOT	Lot number		