Manual

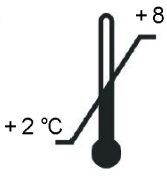
Glycine ELISA Kit

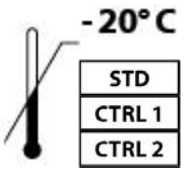
For the determination of glycine in human EDTA plasma and urine

For research use only

Valid from 12.08.2011

K 7013





1. INTENDED USE

The Glycine ELISA Kit is intended for the quantitative determination of glycine in human EDTA plasma and urine. It is for research use only.

2. INTRODUCTION

Glycine is the smallest α-amino acid and it is not essential to the human diet. Most proteins incorporate only small quantities of glycine. A notable exception is collagen, which contains about 35% glycine.

Glycine plays a key role in different metabolic pathways. Porphyrins are biosynthesized from glycine and succinyl-CoA. They are part of Hemoglobin and many enzymes as Catalase and the enzymes of the respiratory chain. Moreover, glycine provides the central C_2N subunit of all purines (components of the DNA).

Glycine is one of three amino acids of glutathione, which is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and the detoxification of many xenobiotics (foreign compounds) and carcinogens.

Treatment with glycine is likely to show diverse beneficial effects: Patients with Type 2 diabetes treated with glycine had a significant decrease in A1C and in proinflammatory cytokines and also an important increase of IFN-gamma. This means that glycine may help to prevent tissue damage caused by chronic inflammation in patients with Type 2 diabetes.

In the central nervous system glycine acts as an inhibitory neurotransmitter, especially in the spinal cord, brainstem, and retina. Inhibitory spinal neurons that release glycine act on alpha motoneurons and decrease activity of skeletal muscles. High concentrations of glycine seem to improve sleep quality.

In the forebrain glycine is a required co-agonist along with glutamate for NMDA receptors. NMDA receptors are excitatory and play a critical role in synaptic plasticity, a cellular mechanism for learning and memory.

Recent study shows that treatment with glycine may help patients with obsessive compulsive disorder.

In patients with schizophrenia glycine serum levels were negatively associated with the intensity of negative symptoms suggesting a possible implication of NMDA receptor dysfunction in pathogenesis of negative symptoms in schizophrenia.

Indications:

In patients with obsessive compulsive disorders and in patients with schizophrenia significantly lower serum levels of glycine have been found.

3. PRINCIPLE OF THE TEST

This assay is based on the method of competitive enzyme linked immunoassays. The sample preparation includes the addition of a derivatization reagent for glycine coupling. Afterwards, the treated samples and the polyclonal glycine antiserum are incubated in wells of microplate coated with a glycine-derivative (tracer). During the incubation period, the target glycine in the sample competes with the tracer immobilized on the wall of the microtiter wells for the binding of the polyclonal antibodies. The glycine in the sample displaces the antibodies out of the binding to the tracer. Therefore, the concentration of the tracer-bound antibody is inverse proportional to the glycine concentration in the sample. During the second incubation step, a peroxidase-conjugated antibody is added to each microtiter well to detect the anti-glycine antibodies. After washing away the unbound components tetramethylbenzidine (TMB) is added as a peroxidase substrate. Finally, the enzymatic reaction is terminated by an acidic stop solution. The color changes from blue to yellow and the absorbance is measured in the photometer at 450 nm. The intensity of the yellow color is inverse proportional to the glycine concentration in the sample; this means, high glycine concentration in the sample reduces the concentration of tracer-bound 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 standards. Glycine present in the patient samples is determined directly from this curve.

4. MATERIAL SUPPLIED

Catalog No	Content	Kit Components	Quantity
K7013MTP	PLATE	One holder with precoated strips	12 x 8 wells
K7013ST	STD	Standards (0, 50, 100, 250, 500, 1000 µM), diluted in reaction buffer (ready to use)	6 x 1 vial
K7013KO	CTRL 1 CTRL 2	Controls, diluted in reaction buffer (ready to use)	2 x 1 vial
K7013WP	WASHBUF	Wash buffer concentrate (10-fold)	2 x 100 ml
K7013AK	AB	Anti-glycine antibody (lyophilized)	2 x 1 vial
K7013K	2.AB	POD antibody (concentrate)	60 µl
K7013CSP	2.ABDIL	Conjugate stabilizing buffer	12 ml
K7013RP	REABUF	Reaction buffer	45 ml
K7013DR	DER	Derivatization reagent	2 x 25,7 mg
K7013LM	DMF	Dimethylformamide (DMF)	3,5 ml
K7013AP	ASYBUF	Assay buffer concentrate (10-fold)	2 x 5 ml
K7013TMB	SUB	TMB substrate	25 ml
K7013AC	STOP	Stop solution	15 ml

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Double distilled water (aqua bidest.)
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- 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 450 nm
  (reference wave length 620 or 690 nm)

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.

- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- Dilute the **wash buffer concentrate (WASHBUF)** with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals may occur due to high salt concentration in the stock solution. The crystals must be redissolved at room temperature or at 37°C using a water bath before dilution. The **WASHBUF** is stable at **2-8°C** until the expiry date stated on the label. **Diluted buffer solution** can be stored in a closed flask at **2-8°C for one month**.

- **Standards (STD) and controls (CTRL1, CTRL2)** are already diluted in **reaction buffer (READBUF)**. Store standards and controls frozen at -20°C, thaw before use in the test, and re-freeze immediately after use. Standards and controls can be re-frozen up to 3 times.

- Dissolve the content of one vial of **derivatization reagent (DER) (25.7 mg)** in **1.5 ml DMF.** CAUTION: DMF is toxic (see chapter 6 - precautions). Put the vial on a horizontal shaker for 5 min. Dispose of any rest of the reagent after use. DER must be **prepared immediately before use**. The ELISA kit can be separated into two performances by providing two DER vials. Please note: **DMF attacks all plastics but not polypropylene products and laboratory glass.**

- Dilute the **assay buffer (ASYBUF)** with aqua bidest. **1:10** before use (5 ml concentrate + 45 ml aqua bidest.), mix well. The ELISA kit can be separated into two performances by providing 2 x 5 ml assay buffer.

- Dissolve the **anti-glycine antibody (AB)** in **3 ml of diluted wash buffer**. The ELISA kit can be separated into two performances by providing two AB vials. **Diluted anti-glycine antibody is stable over a longer period. It can be stored at 2-8°C for 4 weeks.**

- Dilute the **POD antibody (2.AB) 1:200** with **conjugate stabilizing buffer (2.ABDIL)** (e.g. 55 µl 2.AB + 11 ml 2.ABDIL, prepare only the required amount). The undiluted POD antibody (2.AB) is stable at **2-8°C** until the expiry date stated on the label. **Diluted POD antibody (2.AB) is not stable over a longer period. It can be stored at 2-8°C for only 5 days.**

- All other test reagents are ready for use. Test reagents are stable until the expiry date (see label of test package) when stored at 2-8°C.

7. PRECAUTIONS

- For research use only.
- Human materials used in kit components were tested and found to be negative for HIV and Hepatitis B. However, for safety reasons all kit components should be treated as if potentially infectious.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even 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 out immediately with copious quantities of water.
- DMF is toxic. It may cause harm to the unborn child and is harmful by inhalation and in contact with skin. Work under hood and apply preventive skin protection. In case of skin or eye contact flush with plenty of water and get medical attention
- Reagents should not be used beyond the expiration date shown on kit label.

8. SPECIMEN COLLECTION AND PREPARATION

EDTA plasma and urine

- Venous fasting blood and urine are suited for this test system. Blood samples are stable for one week at 2-8°C. Urine samples should be sent cooled, but they are stable for 24 h at room temperature. Otherwise they are stable for two days at 2-8°C. For longer storage, blood and urine samples should be frozen at -20°C.
- Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.
- The EDTA plasma and urine samples are diluted for derivatization.

  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.
- For sample preparation, a DER (derivatization reagent) for coupling of glycine is added (details are given in the sample preparation procedure).

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.

Sample preparation procedure

Dilute **EDTA plasma samples** with reaction buffer by **factor 1:20**,
i.e. 25 µl EDTA plasma sample + 475 µl REABUF (reaction buffer).

Dilute **urine samples** with reaction buffer by **factor 1:100**,
i.e. 10 µl urine sample + 990 µl REABUF (reaction buffer).

Coupling of standards (STD), controls (CTRL) and diluted samples (SAMPLE) is carried out in single analysis (in vials with capacity of at least 2 ml).

1.	Bring all reagents and samples to room temperature (18-26°C).
2.	Add **200 µl of ready to use standards (STD), 200 µl of ready to use controls (CTRL)** and **200 µl of diluted samples (SAMPLE)** in the corresponding vial.
3.	Add **50 µl** of freshly prepared **derivatization reagent (DER)** into each vial (standards, controls and samples), mix well and incubate **for 60 min on a shaker (180-240 rpm) at room temperature (18-26°C)**.
4.	Afterwards add **1500 µl of assay buffer (ASYBUF)** into each vial, mix well and incubate for **30 min on a shaker (180-240 rpm) at room temperature (18-26°C)**.

2 x 50 µl of each treated sample (STD, CTRL, SAMPLE) are used in the ELISA as duplicates.

Test procedure

5.	Mark the positions of standards (STD)/ controls (CTRL)/ samples (SAMPLE) in duplicate on a protocol sheet.
6.	Take as many microtiter strips (PLATE) as needed from kit. Store unused strips covered at 2-8°C. Strips are stable until the expiry date stated on the label.
7.	Wash each well **5 times** by dispensing **250 µl of diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution.
8.	For the analysis in duplicate, take **2 x 50 µl of standards (STD) / controls (CTRL) / samples (SAMPLE)** out of the vial and add into the respective wells of the microtiter plate (PLATE).
9.	Add **50 µl** diluted **anti-glycine antibody (AB)** into each well. Cover the plate tightly.
10.	Incubate overnight **(15-20 hours) at 2-8°C.**
11.	Aspirate the contents of each well. Wash each well **5 times** by dispensing **250 µl of diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution.
12.	Add **100 µl** diluted **POD antibody (2. AB)** into each well.
13.	Cover plate tightly and incubate for **1 hour at room temperature (18-26°C)** on a horizontal shaker (180-240 rpm).
14.	Aspirate the contents of each well. Wash each well **5 times** by dispensing **250 µl of diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution.
15.	Add **100 µl** of **TMB substrate (SUB)** into each well.

16. Incubate for **15-20 min at room temperature (18-26°C)** in the dark*.
17. Add **100 µl of stop solution (STOP)** into each well, mix thoroughly.
18. Determine **absorption** immediately with an ELISA reader at **450 nm**. If the highest extinction of the standards **(STD)** is above the range of the photometer, absorption must be measured immediately at **405 nm** and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e.g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used.

* The intensity of the color change is temperature sensitive. We recommend to observe the color change and to stop the reaction upon good differentiation.

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, samples, and with correct sample treatment), standards, controls, and samples are equally diluted. Therefore, **no dilution factor is required for the calculation of results from serum samples.**

For **urine samples** with 1:100 dilution, the values calculated from the calibration curve **have to be multiplicated by a factor of 5** to obtain the true 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 optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e.g. 0.01).

2. *Point-to-point-calculation*

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

3. *Spline-algorithm*

   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e.g. 0.01).

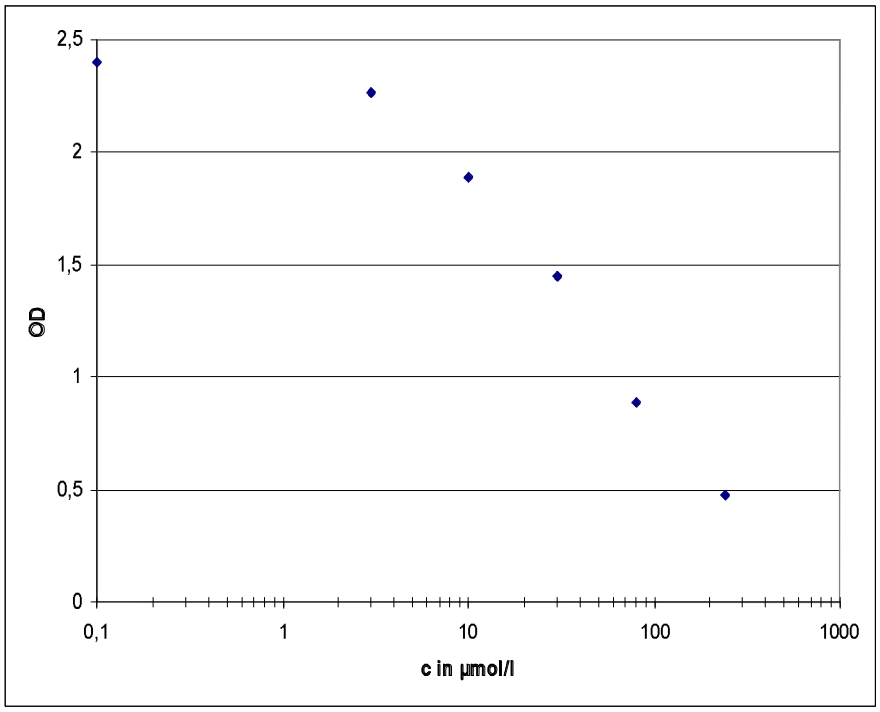
Plausibility of the measured pairs of values should be examined before automatically evaluating the results. If this option is not available within the used program the pairs of values should be controlled manually.

Controls

Control samples or plasma pools 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, do not use it for the calculation of your results.

Example of calibration curve



Expected results

Based on internal studies with plasma samples of evidently healthy persons (n=75) a median of **239,54 µmol/l** was calculated. The normal range was set between the 5th and 95th percentile:

Normal range: **147.55 µmol/l** **438.12 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.

11. PERFORMANCE CHARACTERISTICS

Cross reactivity

L-Alanine	< 1 %
β-Alanine	< 0.2 %

Precision and reproducibility

EDTA plasma:

Intra-Assay (n=9)		
Sample	Glycine [µmol/l]	coefficient of variation (CV) [%]
1	95.4	8.6
2	208.1	11.0

Inter-Assay (n=6)		
Sample	Glycine [µmol/l]	coefficient of variation (CV) [%]
1	124.0	6.6
2	318.6	16.3

Sensitivity

The sensitivity was set as B_0 + 2SD. The zero-standard was measured 32 times.

Sample	Glycine mean value [OD]	2 x Standard deviation (SD)	Detection limit [µmol/l]
zero-standard	1.7	0.2	14.3

Recovery

One sample was spiked with different glycine concentrations and measured using this assay. The analytical recovery rate was determined by the expected and measured glycine levels. The expected levels were calculated as the sum of the measured glycine concentration in the original sample and the spiked glycine amount. The mean recovery rate for all concentrations was 106.1 % (n=10).

EDTA plasma:

Spike [µmol/l]	Glycine expected [µmol/l]	Glycine measured [µmol/l]	Recovery [%]
0	x	X= 167.2	100.0
100	167.2+100= 267.2	276.0	103.3
300	167.2+300= 467.2	537.4	115.0

Linearity

The linearity of the ELISA was determined by the dilution of a spiked patient sample. The mean linearity was 98.3 % (n=10).

EDTA-plasma

Dilution	Measured [µmol/l]	Expected [µmol/l]	Recovery [%]
Original	248.8	248.8	100.0
1+1	118.3	124.4	95.1
1+2	80.9	82.9	97.5
1+3	62.6	62.2	100.7

12. LIMITATIONS

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

13. REFERENCES

- Hons J, Zirko R, Ulrychova M, Cermakova E, Doubek P, Libiger J. Glycine serum level in schizophrenia: relation to negative symptoms. Psychiatry Res. 2010 Apr 30;176(2-3):103-8. Epub 2010 Jan 22
- Ohnuma T, Sakai Y, Maeshima H, Hatano T, Hanzawa R, Abe S, Kida S, Shibata N, Suzuki T, Arai H. Changes in plasma glycine, L-serine, and D-serine levels in patients with schizophrenia as their clinical symptoms improve: results from the Juntendo University Schizophrenia Projects (JUSP). Prog Neuropsychopharmacol Biol Psychiatry. 2008 Dec 12;32(8):1905-12. Epub 2008 Sep 16.
- Greenberg WM, Benedict MM, Doerfer J, Perrin M, Panek L, Cleveland WL, Javitt DC. Adjunctive glycine in the treatment of obsessive-compulsive disorder in adults. J Psychiatr Res. 2009 Mar;43(6):664-70. Epub 2008 Nov 30.
- Ehrlich S, Franke L, Schneider N, Salbach-Andrae H, Schott R, Craciun EM, Pfeiffer E, Uebelhack R, Lehmkuhl U. Aromatic amino acids in weight-recovered females with anorexia nervosa. Int J Eat Disord. 2009 Mar;42(2):166-72.
- Yang CR, Svensson KA. Allosteric modulation of NMDA receptor via elevation of brain glycine and D-serine: the therapeutic potentials for schizophrenia. Pharmacol Ther. 2008 Dec;120(3):317-32. Epub 2008 Aug 27.
- Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Horowitz A, Kelly D. Double-blind, placebo-controlled, crossover trial of glycine adjuvant therapy for treatment-resistant schizophrenia. Br J Psychiatry. 1996 Nov;169(5):610-7.
- Cruz M, Maldonado-Bernal C, Mondragón-Gonzalez R, Sanchez-Barrera R, Wacher NH, Carvajal-Sandoval G, Kumate J. Glycine treatment decreases proinflammatory cytokines and increases interferon-gamma in patients with type 2 diabetes. J Endocrinol Invest. 2008 Aug;31(8):694-9.
- Garcia-Macedo R, Sanchez-Muñoz F, Almanza-Perez JC, Duran-Reyes G, Alarcon-Aguilar F, Cruz M. Glycine increases mRNA adiponectin and diminishes pro-inflammatory adipokines expression in 3T3-L1 cells. Eur J Pharmacol. 2008 Jun 10;587(1-3):317-21. Epub 2008 Apr 8.

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.
- 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


Contains sufficient for <n> tests


Manufacturer


Use by

LOT Lot number