
Instructions for use

Glycine ELISA

Enzyme Immunoassay for the determination of Glycine in urine.
For research use only, not for use in diagnostic procedures.

Catalog Number: MBS495083 (96T)
Storage: 2-8°C

Glycine Urine ELISA

1. **Intended use and principle of the test**

Enzyme Immunoassay for the determination of Glycine in urine. For research use only, not for use in diagnostic procedures.

After derivatization Glycine is determined by ELISA.

The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.

Determination of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

2. **Precautions, Guidelines and Warnings**

- This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
- Reagents of this kit which contain human serum or plasma have been tested and confirmed negative for HIV 1/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- The principles of Good Laboratory Practice (GLP) have to be followed.
- In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
- All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
- Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
- Incubation times do influence the results. All wells should be handled in the same order and time intervals.
- To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
- A calibrator curve must be established for each run.
- The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
- Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
- Avoid contact with Stop Solution containing 0.25 M H₂SO₄. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
- Some reagents contain sodium azide (NaN₃) as preservatives. In case of contact with eyes or skin, rinse off immediately with water. NaN₃ may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with a large volume of water to avoid azide build-up.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets (MSDS). The Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
- Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

3. **Storage and stability**

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiration date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4. Materials

4.1 Contents of the kit

	<u>Symbol</u>	<u>Reagent</u>	<u>Content</u>	<u>Colour Code</u>	
BA D-0024	REAC-PLATE	Reaction Plate	1 x 96 wells		ready for use
BA D-0090	FOILS	Adhesive Foil	2 x 4		ready for use
BA E-0030	WASH-CONC 50x	Wash Buffer Concentrate	1 x 20 ml	light purple	concentrated
BA E-0040	CONJUGATE	Enzyme Conjugate	1 x 12 ml	red	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	Substrate	1 x 12 ml	black	ready for use, containing a solution of TMB
BA E-0080	STOP-SOLN	Stop Solution	1 x 12 ml	light grey	ready for use
BA E-2413	ASSAY-BUFF	Assay Buffer	1 x 20 ml	yellow	ready for use
BA E-2428	EQUA-REAG	Equalizing Reagent	1 x	brown	lyophilized
BA E-2446	D-REAGENT	D-Reagent	1 x 4 ml		ready for use
BA E-2101	STANDARD A	Standard A	1 x 4 ml	white	ready for use
BA E-2102	STANDARD B	Standard B	1 x 4 ml	light yellow	ready for use
BA E-2103	STANDARD C	Standard C	1 x 4 ml	orange	ready for use
BA E-2104	STANDARD D	Standard D	1 x 4 ml	dark blue	ready for use
BA E-2105	STANDARD E	Standard E	1 x 4 ml	light grey	ready for use
BA E-2106	STANDARD F	Standard F	1 x 4 ml	black	ready for use
BA E-2110	AS GLY	Glycine Antiserum	1 x 6 ml	blue	ready for use, from rabbit, blue coloured
BA E-2131	W GLY	Glycine Microtiter Strips	1 x 96 wells		12 strips, 8 wells each, break apart, pre-coated
BA E-2129	RED-CONC 100x	Reducing Concentrate	1 x 1 ml	pink	concentrated
BA E-2151	CONTROL 1	Control 1	1 x 4 ml	light green	ready for use
BA E-2152	CONTROL 2	Control 2	1 x 4 ml	dark red	ready for use

4.2 Additional materials and equipment required but not provided with the kit

- Calibrated precision pipettes to dispense volumes between 20 µl – 300 µl; 2,5 ml; 12,5 ml
- Polystyrene or polypropylene tubes and suitable rack
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm (reference wavelength 620 - 650 nm)
- Shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)
- Vortex mixer

5. Sample collection and storage

Urine

Spontaneous or 24-hour urine, collected in a bottle containing 10 - 15 ml of 6 M HCl, should be used. Storage: up to 48 hours at 2 - 8 °C or for a longer period (up to 6 months) at -20 °C. More than 3 freeze-thaw cycles should be avoided.

6. Test procedure

Allow all reagents to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended.

6.1 Preparation of reagents

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.

Storage: up to 6 months 2 - 8 °C.

Equalizing Reagent

Reconstitute the Equalizing Reagent with **12.5 ml** of **Assay Buffer**.

Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots at -20 °C and may be thawed only once.


Reducing Solution

Dilute **Reducing Concentrate** 1: 100 with **water** (deionized, distilled, or ultra-pure) and mix thoroughly. Use immediately!


Examples for the preparation of Reducing Solution:

Reducing Concentrate	40 µl	50 µl	80 µl	160 µl
Water	3,96 ml	4,95 ml	7,92 ml	15,84 ml

6.2 Dilution

1.	Pipette 20 µl of standards, controls and samples into the respective tubes .
2.	Add 2,5 ml of water (deionized, distilled, or ultra-pure) to all tubes and mix thoroughly (vortex).
	Take 100 µl for the derivatization .

6.3 Derivatization

1.	Pipette 100 µl of the diluted standards, controls and samples into the appropriate wells of the Reaction Plate .
2.	Add 50 µl of the Equalizing Reagent to all wells.
4.	Add 10 µl of the D-Reagent to all wells.
5.	Cover plate with Adhesive Foil and shake for 2 h at RT (20 - 25 °C) on a shaker (approx. 600 rpm).
6.	Pipette 150 µl Reducing Solution (refer to 6.1) into all wells. The Reducing Solution should be prepared directly prior to use!
7.	Shake for 30 min at RT (20 - 25 °C) on a shaker (approx. 600 rpm).
	Take 25 µl for the ELISA!

6.4 Glycine ELISA

1.	Pipette 25 µl of the prepared standards, controls and samples into the appropriate wells of the Glycine Microtiter Strips .
2.	Pipette 50 µl of the Glycine Antiserum into all wells and mix shortly.
3.	Cover plate with Adhesive Foil and incubate for 15 - 20 h (overnight) at 2 - 8 °C .
4.	Remove the foil. Discard or aspirate the contents of the wells. Wash the plate 3 x by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
5.	Pipette 100 µl of the Enzyme Conjugate into all wells.
6.	Incubate for 30 min at RT (20 - 25 °C) on a shaker (approx. 600 rpm).
7.	Remove the foil. Discard or aspirate the contents of the wells. Wash the plate 3 x by adding 300 µl of Wash Buffer , discarding the content and blotting dry each time by tapping the inverted plate on absorbent material.
8.	Pipette 100 µl of the Substrate into all wells and incubate for 20-30 min at RT (20 - 25 °C) on a shaker (approx. 600 rpm). Avoid exposure to direct sunlight!
9.	Add 100 µl of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the standards					
Standard	A	B	C	D	E	F
Glycine (µg/ml)	0	10	30	100	300	1 000

The calibration curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis). Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

The concentrations of the **samples** and **controls** can be read directly from the standard curve.

7.1 Quality control

It is recommended to use control samples according to national regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC-Report.

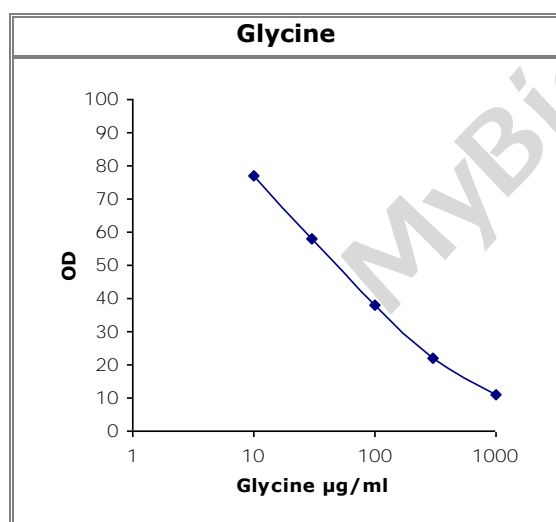
7.2 Calibration

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20 - 25 °C.

In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm

7.3 Typical calibration curve

⚠ Example, do not use for calculation!



8. Assay characteristics


Analytical Sensitivity (Limit of Detection)	Glycine
	3,3 µg/ml

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
		Glycine
	Glycine	100
	D-Serin	3,7
	L-Cystein	1,8
	Beta-Alanin	0,7
	GABA	0,8
	L-Aspartic Acid	<0,1
	L-Glutamat	<0,1
	Taurin	<0,1

Precision					
Intra-Assay			Inter-Assay		
Sample	Range (µg/ml)	CV (%)	Sample	Range (µg/ml)	CV (%)
1 (n = 20)	66,7 ± 4,2	6,2	1 (n = 23)	63,3 ± 7,9	13
2 (n = 20)	94,0 ± 3,5	3,7	2 (n = 23)	91,1 ± 9,2	10
3 (n = 20)	217 ± 11,0	5,1	3 (n = 23)	211 ± 9,4	9,4

Linearity		Range Linearity %	Serial dilution up to	Mean Linearity %
	Urine	94 - 116	1:128	100

Recovery		Mean Creatinine (mg/dl)	Mean Recovery (%)	Mean Range (%)
	Sample 1	38,8	108	96 - 116
	Sample 2	102	95	93 - 96
	Sample 3	135	108	105 - 114

 **For updated literature, information about clinical significance or any other information please contact your local supplier.**