Manufactured for Immuno-Biological Laboratories Inc.
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Instructions for use

Glycine ELISA

Enzyme Immunoassay for the determination of Glycine in urine.

For research use only, not for use in diagnostic procedures.

REF **IB89191**

Σ 96

+2 +8 °C

RUO For Research use only- Not for use in diagnostic procedures

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. Procedural Cautions, Guidelines and Warnings

(1) 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.
(2) The principles of Good Laboratory Practice (GLP) have to be followed.
(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable latex gloves and protective glasses where necessary.
(4) 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.
(5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(6) The microtiter plate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(8) 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.
(9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(11) A standard curve must be established for each run.
(12) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(13) 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.
(14) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(15) 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.
(16) For information on hazardous substances included in the kit please refer to Material Safety Data Sheet (MSDS). The Material Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(17) Kit reagents must be regarded as hazardous waste and disposed according to national regulations.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C. Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Content of the kit

BA D-0090	FOILS	**Adhesive Foil** - Ready to use
Content:	Adhesive Foils in a resealable pouch	
Volume:	1 x 4 foils	

BA E-0030 WASH-CONC 50x **Wash Buffer Concentrate -** Concentrated 50x

Content: Buffer with a non-ionic detergent and physiological pH

Volume: 1 x 20 ml/vial, light purple cap

BA D-0024 REAC-PLATE **Reaction Plate** - Ready to use

Content: 1 x 96 well plate, empty in a resealable pouch

BA E-0040 CONJUGATE **Enzyme Conjugate** - Ready to use

Content: Goat anti-rabbit immunoglobulins conjugated with peroxidase

Volume: 1 x 12 ml/vial, red cap

BA E-0055 SUBSTRATE **Substrate** - Ready to use

Content: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide

Volume: 1 x 12 ml/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use

Content: 0.25 M sulfuric acid

Volume: 1 x 12 ml/vial, light grey cap

BA E-2413 ASSAY-BUFF **Assay Buffer** - Ready to use

Contents: Buffer with alkaline pH

Volume: 1 x 20 ml/vial, yellow cap

BA E-2428 EQUA-REAG **Equalizing Reagent** - Lyophilized

Contents: Lyophilized protein

Volume: 1 vial, brown cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration µg/ml	Concentration µmol/l	Volume/ Vial
BA E-2101	STANDARD A	white	0	0	4 ml
BA E-2102	STANDARD B	light yellow	10	133	4 ml
BA E-2103	STANDARD C	orange	30	399	4 ml
BA E-2104	STANDARD D	dark blue	100	1 330	4 ml
BA E-2105	STANDARD E	light grey	300	3 990	4 ml
BA E-2106	STANDARD F	black	1 000	13 300	4 ml
BA E-2151	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA E-2152	CONTROL 2	dark red			4 ml

Conversion: Glycine (µg/ml) x 13.3 = Glycine (µmol/l)

Contents: Acidic buffer with non-mercury preservative, spiked with defined quantity of Glycine

BA E-2131 MTP GLY **Glycine Microtiter Strips** - Ready to use

Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable foil pouch with desiccant

BA E-2110 AS GLY **Glycine Antiserum** - Ready to use

Contents: Rabbit anti- glycine antibody, blue coloured

Volume: 1 x 6 ml/vial, blue cap

BA E-2446	D-REAGENT	**D-Reagent - Ready to use**
Contents:	Crosslinking agent in dimethylsulfoxide	
Volume:	1 x 4 ml/vial, white cap	
Hazards identification:		

H318 Causes serious eye damage.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H332 Harmful if inhaled.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.

BA E-2129	RED-CONC 100x	**Reducing Concentrate** - Ready to use
Contents:	Reducing agent in sodium hydroxide	
Volume:	1 x 6 ml/vial, pink cap	

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 and if possible 620 - 650 nm
- Microtiter plate 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.
Determine the total volume of urine excreted during a period of 24 h for calculation of the results.
Storage: for longer periods (up to 6 months) at -20 °C.
Repeated freezing and thawing should be avoided.
Avoid exposure to direct sunlight.

6. Test procedure

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

The binding of the antibodies and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the absorption values may vary if a thermostat is not used. The higher the temperature, the higher the absorption values will be. The absorption values also depend on 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.*

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: 1 month at 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 for max 1 month at -20 °C and may be thawed only once.

D-Reagent
The D-Reagent has a freezing point of 18.5 °C. To ensure that the D-Reagent is liquid when being used, it must be ensured that the D-Reagent has reached room temperature and forms a homogeneous, crystal-free solution.

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** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	**Glycine**
	3.3 – 1 000 µg/ml

The standard 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).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

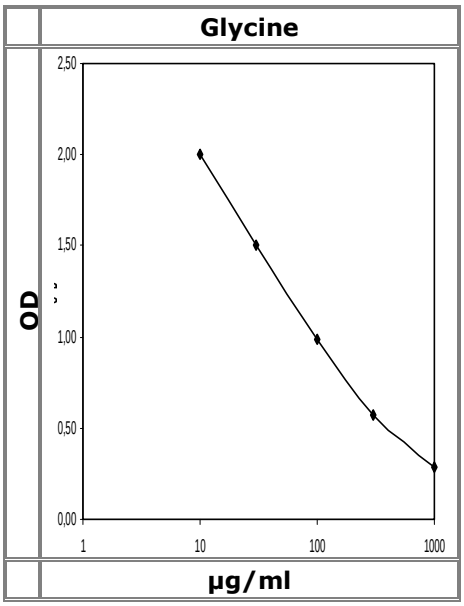
The concentrations of the **samples** and **controls** can be read directly from the standard curve.

7.1 Quality control

The confidence limits of the kit controls are indicated on the QC-Report.

7.2 Typical standard 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

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

		Mean Creatinine (mg/dl)	Mean Recovery (%)	Range Recovery (%)
Recovery	Sample 1	38.8	108	96 – 116
	Sample 2	102	95	93 – 96
	Sample 3	135	108	105 – 114

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⚠ ***For literature or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!