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LDN®

Instructions for use

Glutamate Research ELISA

REF **BA E-2300**

96

+2 +8 °C

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

Glutamate Research ELISA

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of L-Glutamate in biological samples.

After extraction and derivatisation Glutamate is quantitatively determined by ELISA.
The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated 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.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Storage and stability

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

4.1 Contents of the kit

BA D-0024	REAC-PLATE	**Reaction Plate**	1 x 96 wells	ready for use
BA D-0090	FOILS	**Adhesive Foil**	1 x 4	ready for use
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
BA E-0040	CONJUGATE	**Enzyme Conjugate**	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4
BA E-2401	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA E-2402	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA E-2403	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA E-2404	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA E-2405	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA E-2406	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA E-2410	AS GLUT	**Glutamate Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-2413	ASSAY-BUFF	**Assay Buffer**	1 x 20 mL	ready for use
BA E-2428	EQUA-REAG	**Equalizing Reagent**	1 x	lyophilized
BA E-2431	GLUT	**Glutamate Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, precoated
BA E-2442	EXTRACT-PLATE 48	**Extraction Plate**	2 x 48 wells	ready for use
BA E-2446	D-REAGENT	**D-Reagent**	1 x 4 mL	ready for use
BA E-2451	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA E-2452	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA E-2458	Q-BUFFER	**Q-Buffer**	1 x 20 mL	ready for use
BA E-2460	DILUENT	**Diluent**	1 x 20 mL	ready for use
BA E-2787	NAOH	**NaOH**	1 x 2 mL	ready for use

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

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- Polystyrene tubes and suitable rack
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

5. Sample collection and storage

In general this assay is dedicated for any biological sample such as plasma, serum, tissue homogenates and other samples.
Storage: up to 24 hours at 2 – 8 °C, for longer periods (up to 6 months) at - 20°C.

6. Test procedure

Allow all reagents and samples to reach room temperature. Duplicate determinations are recommended.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with distilled water 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 aliquotes at -20°C and may be thawed only once.

6.2 Preparation of samples

The Glutamate ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to adapt the protocol to his specific needs:

- **Serum/plasma samples** should always be pre-diluted 1:5 (e.g. 100 µL serum/plasma + 400 µL distilled water). Serum values of Glutamate are higher than for urine. The pre-dilution step makes sure that the sample is measured in the linear range of the standard curve. **The results have to be corrected for the dilution factor**.
- Avoid excess of acid: excess of acid might exceed the buffer capacity of the dilution buffer. A **pH of 5.0** during the extraction is mandatory.
- It is advisable to perform a **Proof of Principle** to determine the recovery of glutamate in the samples. Prepare a stock solution of glutamate. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.
- The sample volume determines the sensitivity of this test. Determine the sample volume needed to determine glutamate in your sample by testing different amounts of sample volumes.
- If a sample volume **< 100 µl** is used, dist. water has to be added to a **final volume of 100 µl** and this **prediluted sample** has to be used for the extraction procedure (please refer to point 6.3 of this protocol). This sample predilution has to be considered in the calculation of results (please refer to point 7 of this protocol).

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

6.3 Extraction

1.	Pipette **100 µL** of the **standards, controls** and **samples** (diluted or undiluted) into the appropriate wells of the **Extraction Plate.**
2.	Add **100 µL** of the **Diluent** to all wells. Cover plate with **Adhesive Foil** and **shake** for **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
3.	Use **25 µl** for the subsequent derivatization!

6.4 Derivatization

1.	Pipette **25 µL** of the **extracted standards, controls** and **samples** into the appropriate wells of the **Reaction Plate.**
2.	Pipette **10 µL** of **NaOH** into all wells.
3.	Pipette **50 µL** of the **Equalizing Reagent** into all wells.
4.	Pipette **10 µL** of the **D-Reagent** into all wells.
5.	Cover plate with **Adhesive Foil** and shake for **2 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6.	Pipette **75 µL** of the **Q-Buffer** into all wells.
7.	Shake for **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
8.	**Use 25 µl for the ELISA!**

6.5 Glutamate ELISA

1.	Pipette **25 µL** of the **prepared standards, controls and samples** into the appropriate wells of the **Glutamate Microtiter Strips.**
2.	Pipette **50 µL** of the **Glutamate Antiserum** into all wells and mix shortly.
3.	Cover plate with **Adhesive Foil** and incubate for **15 - 20 hours** (overnight) at **2 – 8 °C**. ***Alternatively*** *incubate* ***2 hours*** *at* ***RT*** *(20-25°C) on a* ***shaker*** *(approx. 600rpm).*
4.	Remove the foil and discard. Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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.	Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry 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 sun light!***
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**
Glutamate (µg/mL)	0	0.6	2	6	20	60
Glutamate (µmol/L)	0	4.08	13.6	40.8	136	408
Conversion:	Glutamate (µg/mL) x 6.8 = Glutamate (µmol/L)					

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).

Controls and undiluted samples:
The concentrations of undiluted samples and controls can be read directly from the standard curve.

Diluted samples
If only a sample volume **< 100 µl** was used for the extraction, the concentration read from the standard curve has to be **multiplied** with a **volume-factor**:

$$\textbf{Volume-factor} = \frac{\textbf{100 µl}}{\textbf{used sample volume (µl)}}$$

For example, plasma and serum samples should be diluted before the extraction procedure 1:5 with distilled water. In that case the volume factor is 5 (100 µl/20 µl) and the read concentration has to be multiplied with 5.

7.1 Quality control
It is recommended to use control samples according to state and federal 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.

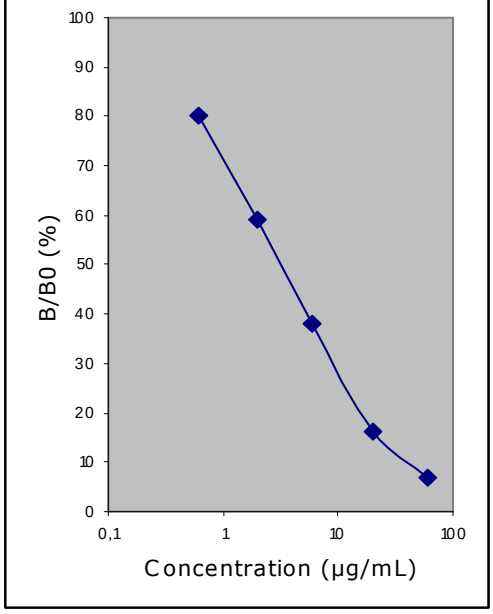
7.2 Calibration

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

Glutamate



⚠ *Example, do not use for calculation!*

8. Assay characteristics

Analytical Sensitivity (Limit of Detection)	**Glutamate**
	0.3 µg/mL

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**
	Glutamate	100
	Glutamine	< 0.01
	Aspartate	0.09
	Glycine	< 0.01
	Alanine	< 0.01
	5-aminovaleric acid	< 0.01

Precision					
Intra-Assay			**Inter-Assay**		
Sample	Range (µg/mL)	CV (%)	Sample	Range (µg/mL)	CV (%)
1 (n = 25)	6.9 ± 0.5	7.3	1 (n = 19)	0.8 ± 0.18	19
2 (n = 25)	16.0 ± 1.0	6.3	2 (n = 19)	9 ± 1.2	13

Linearity		Range	Serial dilution up to	Range (%)
	Glutamate (serum)	1 – 24 µg/mL	1:20	82 - 98

Recovery		Mean (%)	Range (%)	% Recovery after spiking
	Glutamate (serum)	99	96 - 104	

⚠ ***For current literature, information about clinical significance 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!