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LDN®

Instructions for use

Glutamate Food ELISA

REF FC E-3700

96

+2 +8 °C

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

Glutamate ELISA

1. Intended use and principle of the test

Enzyme immunoassay for the quantitative determination of L-Glutamate in food.

After 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 absorbances 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 (e.g. GLP). 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 complaint 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 protocol can affect the results . 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

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.Optimal test results are only obtained when using calibrated pipettes.

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 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-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-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
FC E-3710	AS GLUT	**Glutamate Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap

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 preparation

The following protocol refers to the preparation of soup samples. For any other kind of food sample please contact the manufacturer directly to receive a protocol for sample preparation.

Extraction

- homogenize 2 g of instant soup in 100 ml of hot water (+/- 70 – 80°C) and incubate for 10 minutes.
- let the soup cool down to room temperature.
- add distilled water to a final volume to 250 mL.
- filter the homogenate through folded filter paper (S595)

Dilution

- dilute the filtrate 1:25 with distilled water (for example 100µL filtrate + 2.4mL distilled water)

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 Derivatization

1.	Pipette **25 µL** of the **standards, controls** and **diluted samples** into the appropriate wells of the **Reaction Plate.**
2.	Pipette **50 µL** of the **Equalizing Reagent** into all wells and mix shortly.
4.	Pipette **10 µL** of the **D-Reagent** into all wells.
5.	Shake for **90 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6.	Pipette **100 µL** of the **Q-Buffer** into all wells.
7.	Shake for **5min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
8.	Use **25 µl** for the subsequent ELISA

6.3 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.
3.	Cover plate with **Adhesive Foil** and incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
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 **15 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 **15 ± 2 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).

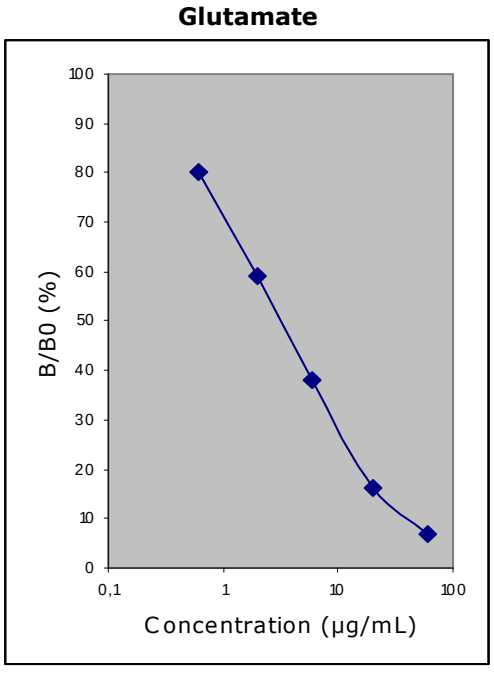
Soup samples
The read concentrations of **soup samples** have to be **multiplied by 25.**

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



⚠ *Example, do not use for calculation!*

8. Assay characteristics

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
Analytical Sensitivity (Limit of Detection)	Glutamate	0.3 µg/mL	Mean signal (Zero-Standard) - 2SD

Precision			
Intra-Assay (n = 10)		**Inter-Assay**(n = 5)	
Range (µg/mL)	CV (%)	Range (µg/mL)	CV (%)
97 ± 2.5	2.6	98 ± 4,9	5

Recovery		Mean (%)	Range (%)	% Recovery after spiking
	Soup	100	97- 102	

⚠ ***For updated 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!