EN

# L-Glutamic acid

**REF 984636**

| | |
|---|---|
| ❶ | 3 x 6 ml Reagent 1 |
| ❷ | 3 x 3 ml Reagent 2 |
| ❸ | 3 x 3 ml Reagent 3 |
| ❹ | 1 x 5 ml Standard |

**INTENDED USE**
Reagent for photometric determination of L-Glutamic acid in homogenous liquid samples using automated Thermo Scientific™ Arena™ or Gallery™ analyzer.

**METHOD**
L-Glutamic acid (L-glutamate) is oxidatively deaminated by nicotinamideadenine dinucleotide (NAD) to 2-oxoglutarate in the presence of the enzyme glutamate dehydrogenase (GIDH).

In the reaction catalyzed by diaphorase the NADH formed converts iodonitrotetrazolium chloride (INT) to a formazan which is measured at its maximum in the visible range at 492 nm. The equilibrium of reaction lies on the side of L-glutamic acid. By trapping the formed NADH with INT, the equilibrium is displaced in favor of 2-oxoglutarate.

$$\text{L-glutamate} + NAD^+ + H_2O \xrightarrow{\text{GLDH}} \text{2-Oxoglutarat} + NADH + NH_4^+$$

$$NADH + JNT + H^+ \xrightarrow{\text{Diaphorase}} NAD^+ + \text{Formazan}$$

**REAGENT INFORMATION**

| | Barcode information |
|---|---|
| Reagent 1 (R1) 3 x 6 ml | A46 |
| Reagent 2 (R2) 3 x 3 ml | A47 |
| Reagent 3 (R3) 3 x 3 ml | A48 |
| Standard solution 1 x 5 ml | |

**Concentrations**

| | | |
|---|---|---|
| **R1** | Potassium phosphate,<br>Triethanolamin,<br>Triton X-100 | pH 8.6 |
| **R2** | Jodnitrotetrazoliumchlorid, JNT<br>NAD | 0.6 g/<br>10 g/l |
| **R3** | Diasporase<br>GLDH | 2.0 g/l<br>1 KU/L |
| **Standard** | L-Glutamic acid solution | 100 mg/l |

**Precautions**
R1 is hazardous.
See separate sheet inside the kit for Hazardous- and Precautions-phrases: H319, P280, P305+P351+P338, P337+P313.

R3 contain sodium azide as preservative.

Exercise the normal precautions required for handling all laboratory reagents.
The product has to be disposed of as laboratory chemical in accordance with local regulations.

**Preparation**
The reagents R1, R2, R3 and Standard solution are ready-to-use.
**Note:** Check that there are no bubbles on the surface of the reagent when you insert vials in the analyzer.

**Storage and Stability**
Reagents in unopened vials are stable at 2…8 °C until the expiry date printed on the label. Do not freeze the reagents.
**Note:** JNT in Reagent 2 is photosensitive. Keep it in dark when not in use.

**SAMPLES**

**Sample Type**
Homogenous liquid samples.

**Sample preparation**
Use clear, colorless and practically neutral liquid samples directly.

If the sample has substances interfering the measurement, please handle it according to the following suitable preparation procedure:

- Filter turbid solutions.
- Cut solid and semi-solid samples and mix thoroughly (electric mixer, meat grinder or mortar) extract with water or dissolve; filter if necessary.
- Degas samples containing carbon dioxide e.g by filtration
- Acidic samples, adjust pH with NaOH/ KOH to approximately pH 8.
- Treat protein containing samples with Perchloric acid
- Treat fat containing samples with hot water extract in a volumetric vial, use temperature above to the melting point of the fat, allow fat to cool and separate, keep 15 minute on ice and filter.

Determination of L-glutamic acid in soy sauce and condiment
Use a diluted sample for the determination.

A suggestion for sample preparation when determining L-glutamic acid in meat extracts, soup or bouillon cubes
Accurately weigh 1 g sample to a beaker and dissolve with 70 ml of deionized water. Heat to 70°C for 10 min and allow to cool to room temperature. Transfer into a 100 ml volumetric flask and fill up to the mark with deionized water. Filter through a filter paper, re-filter if necessary. Dilute the sample if necessary.

A suggestion for sample preparation when determining L-glutamic acid in meat products (sausages)
Weigh 10 g of minced sausage and homogenize with 80 ml of perchloric acid (1 M) for 10 min using a homogenizer. Centrifuge, decant the supernatant, and filter. Discard the first few ml of the filtrate and pipette 20 ml into a beaker, adjust to pH 10.0 with potassium hydroxide (2 M); measure the volume of KOH. To obtain quantitative precipitation of the potassium perchlorate formed, place in an ice-bath or refrigerator for 20 min and filter.

A suggestion for sample preparation when determining L-glutamic acid in vegetable and fruit products
Accurately weigh 1 g of the homogenized sample. Extract with 50 ml of water (for 10 min) and transfer to a 100 ml volumetric flask. Fill up to the mark with water, shake and filter.
Generally the sample needs not to be decolorized. In the case decolorization is required, add 1 % polyamide powder or polyvinylpolypyrrolidone (PVPP) to the sample solution; stir briefly (1 min) and filter.

**Materials required but not provided**
Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

**Calibration**
The standard included in the kit is ready-to-use.

**Quality Control**
Use quality control samples at least once a day and after each calibration and every time a new bottle of reagent is used. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

Available controls:
L-Glutamic acid Standard included in this kit can be used. If that is used also for calibration, an additional internal control is recommended to be used. L-Glutamic acid (e.g. Sigma G1251) is recommended.

**CALCULATION OF RESULTS**
The results are calculated automatically by the analyzer using a calibration curve.



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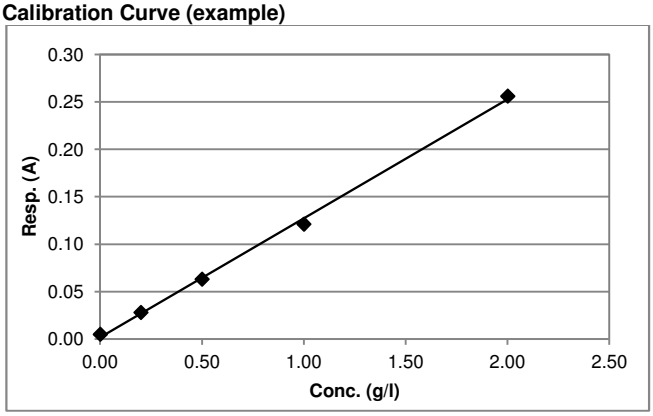
**Calibration Curve (example)**




Note that the calibration curve is lot dependent. This calibration curve was performed by the Gallery analyzer.

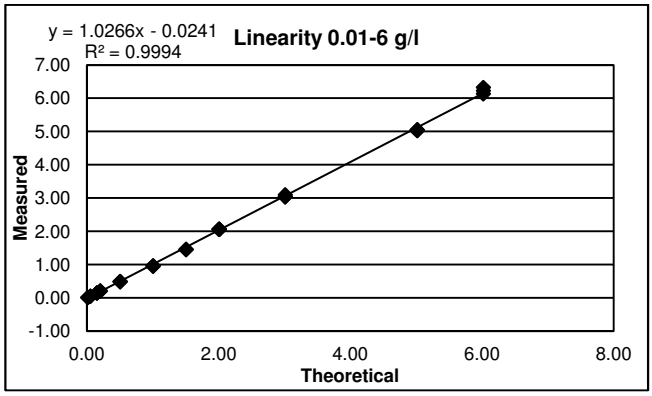
## LIMITATIONS OF THE PROCEDURE

**Interference**
The method is specific for L-Glutamic acid.

## MEASURING RANGE
The test has been developed to determine L-glutamic acid within a measuring range from 0.01 to 6 g/l.

Additionally dillution 1+99 can be used to measure concentrations from 6-10 g/l.

## PERFORMANCE CHARACTERISTICS
The results obtained in individual laboratories may differ from the performance data given. Linearity testing has been performed with water based standard solutions. Different matrixes may change the linearity limits of the test.




**Precision**
**Gallery analyzer**

| | Soy sauce | | Soy sauce | |
|---|---|---|---|---|
| | **N** | 50 | **N** | 50 |
| | **Mean** | 1.82 | **Mean** | 4.24 |
| | **SD** | **CV %** | **SD** | **CV %** |
| Within run | 0.013 | 0.7 % | 0.066 | 1.6 % |
| Between run | 0.026 | 1.4 % | 0.069 | 1.6 % |
| Total | 0.030 | 1.6 % | 0.096 | 2.3 % |

**Determination limit (=Test limit low)**
The determination limit is the lowest concentration that can be measured quantitatively. The determination limit for this method is 0.01 g/l L-glutamic acid.

## OTHER REMARKS
Note that part of the application performance has been verified with pure chemicals dissolved in deionized water. The results obtained in individual laboratories may differ from the given performance data due to e.g. sample matrix, concentrations or analysis environment. Each laboratory is responsible to verify the method to prove the analysis performance.

## WASTE MANAGEMENT
Please refer to local legal requirements. It is recommended to empty the analyzer cuvette waste bin and waste water daily. Emptying should be done immediately after the analysis when using hazardous reagents/solutions.
**Note:** If using reagents/solutions that react with each other, cuvette waste bin and waste water should be emptied and washed between use of these reagents.

## BIBLIOGRAPHY
1) Beutler, H.-O. & Michal, G. (1974) in Methoden der enzymatischen Analyse (Bergmeyer, H. U., Hrsg.) 3. Aufl., Bd. 2, S. 1753-1759, Verlag Chemie, Weinheim, and (1974) in
2) Methods of Enzymatic Analysis (Bergmeyer, H. U., ed.) 2nd ed., vol. 4, pp. 1708-1713, Verlag Chemie, Weinheim/Academic Press, Inc. New York and London

## ADDITIONAL MATERIAL
Certificate of analysis and SDS are available at
www.e-labeling.eu/TSF

Applications for Gallery and Arena automated analyzers are available upon request from the local sales representative. Information in the Application note can change without prior notice.

## MANUFACTURER
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## CONTACT INFORMATION
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**Changes from previous version**
Update



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