Enzymatic UV test for the determination of L-glutamic acid in foodstuffs and other sample materials
Test combination for 50 determinations

For *in vitro* use only
Store between 2 - 8 °C

This test was validated for the following matrices: vegetable broth and bouillon cube, hot dog sauce, vegetable puree, sausage, ketchup, lasagne bolognese, tomato pesto and soy sauce. For detailed results and further information on validation data, please refer to the validation report.

Other foodstuffs or sample materials can be tested and must be validated by the user.

1. Test principle

L-Glutamic acid (L-glutamate) is oxidatively deaminated by nicotinamide adenine dinucleotide (NAD) to 2-oxoglutarate in presence of the enzyme glutamate dehydrogenase (GlDH):

L-Glutamate + NAD^+ + H_2O ← GlDH → 2-oxoglutarate + NADH + NH_4^+

The reaction of L-glutamic acid is quantitative. The equilibrium of the reaction lies on the side of the 2-oxoglutarate. The amount of NADH formed in this reaction is stoichiometric to the amount of L-glutamic acid. NADH is measured on the basis of its specific absorbance at a wavelength of 340 nm. The result is stated in g/L or g/100 g L-glutamic acid.

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing.

With manual processing, the reagents are sufficient for 50 determinations. The number of determinations for automated processing is increased by a multiple, but depends on the device. The incubation times for automated processing may vary and must therefore be verified.

• Reagent 1	2 x 50 mL	Buffer, GlDH
• Reagent 2	2 x 12.5 mL	Buffer, NAD

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the indicated shelf life (see labeling) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

This product/test is only suitable for use within the scope of its intended purpose. The instruction for use must be strictly followed.

General safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

3.1. General

- The sample preparation for manual and automated testing is identical.
- Bring samples to room temperature before measurement.
- Use **liquid, clear and almost neutral** sample solutions directly or after dilution with dist. water to a concentration within the measuring range (see performance data).
- Filter **turbid solutions** using a pleated filter and, if necessary, a syringe filter.
- Degas samples **containing carbonic acid**, e.g. by filtration or centrifugation.
- **Highly colored and highly concentrated** samples should be decolorized with polyvinylpolypyrrolidone (PVPP).
- Clarify **protein-containing** samples with perchloric acid.
- Weigh samples with a **high fat content** into a volumetric flask and extract with hot water; allow the sample solution to cool for fat separation (e.g. 15 min in an ice bath); fill the volumetric flask up to the mark with water and filter before testing.
- Sufficiently homogenize and crush **solid and semi-solid** samples; extract with water or dissolve in dist. water and filter if necessary.
- Adjust **strongly acidic** samples to approx. pH 8.0 by adding sodium or potassium hydroxide solution.

3.2. Determination of L-glutamic acid in soy sauces, hot dog sauce and ketchup

- Dilute samples with dist. water to a concentration range of 10 - 1250 mg/L.

3.3. Determination of L-glutamic acid in meat extracts, instant soup or bouillon cubes

- Accurately weigh approx. 1 g of the sample into a beaker or a 50 mL centrifuge tube.
- Add 10 - 20 mL dist. water and mix.
- Incubate for 10 - 15 minutes in a water bath at 70 °C.
- Transfer the warm suspension to a 100 mL volumetric flask, cool for approx. 15 min in an ice bath and then fill up to the mark with dist. water.
- Filter using a paper filter and repeat the process, if necessary, until a clear solution is obtained.

3.4. Determination of L-glutamic acid in meat products and sausage

- Weigh 10 g of sample accurately into a beaker or a 50 mL centrifuge tube.
- Add 10 - 20 mL dist. water and mix.
- Incubate for 10 - 15 minutes in a water bath at 70 °C.
- Add 1 drop of concentrated sulphuric acid (work under a fume hood if necessary).
- Transfer the warm suspension to a 100 mL volumetric flask, allow to cool at room temperature and, after cooling to 20 - 25 °C, fill up to the mark with dist. water.
- Filter using a pleated filter and, if necessary, a syringe filter; repeat the procedure until a clear solution is obtained.

3.5. Determination of L-glutamic acid in fruits or vegetable products (analogous to the §64 method for tomato paste and ketchup)

- Accurately weigh approx. 1 g of the sample into a 50 mL centrifuge tube and dissolve in 10 - 20 mL dist. water.
- Mix the sample well (e.g. by shaking or vortexing).
- Fill up to approx. 50 mL with dist. water.
- Extract for 10 min on a shaker or roller mixer.
- Transfer the suspension to a 100 mL volumetric flask and fill up to the mark with dist. water.
- Filter using a pleated filter and, if necessary, a syringe filter; repeat the procedure until a clear solution is obtained.

3.6. Further advice

The use of a multistepper pipette is recommended for the addition of reagent 1 and 2. Use a separate tip for each component.

Use separate tips for each sample extract (and the control solutions) to avoid cross-contamination; rinse the tip before pipetting.

4. Assay procedure

Wavelength: 340 nm
Cuvettes: 1 cm light path
Temperature: 20 - 37 °C (during the measurement)
Measuring range: 10 - 1250 mg/L (basic application 100 µL)

	Reagent blank	Samples / controls
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 min at 20 - 37 °C. Read absorbance $\mathbf{A_1}$, then add:		
Reagent 2	500 µL	500 µL
Mix, incubate for 30 min at 20 - 37 °C and measure absorbance $\mathbf{A_2}$.		

The reagent blank (RB) value must also be determined for **each** sample and subtracted from the respective sample result.

The specified incubation times may vary depending on the prevailing laboratory conditions and the pipetting accuracy. It is therefore recommended to wait for the end of the reaction during the first runs and to adjust the times if necessary.

5. Calculation of results

5.1. Calculation of sample solutions

5.1.1. Total concentration of L-glutamic acid

$$\Delta A_{\text{L-Glutamic acid}} = (A_2 - A_1 \times df)_{\text{Sample or control}} - (A_2 - A_1 \times df)_{\text{RB}}$$

df: (Reagent) dilution factor
RB: Reagent blank

$$df_{100\,\mu L} = \frac{\text{sample volume + volume R1}}{\text{total test volume}} = \mathbf{0.808}$$

Stated df of 0.808 applies for a basic application of 100 µL. **Increasing the sample volume** (up to 1000 µL; refer to validation report) with unchanged reagent volumes **requires conversion of the dilution factor (df)**.
If the volume is increased, the test system may be affected. In general, this must be checked depending on the matrix. It is recommended to adjust the sample blank to the increased sample volume.

The concentration of L-glutamic acid is calculated using Lambert-Beer's law:

$$\mathbf{C}_{\text{L-Glutamic acid}}\ \mathbf{[g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.6072 \times \Delta A}\ (\times F)$$

If the sample extract was diluted before measurement, this result has to be multiplied with the **pre-dilution factor F**.

V:	Test volume (basic application) [mL]	= 2.600
MW:	Molecular weight L-glutamic acid [g/mol]	= 147.13
d:	Optical path [cm]	= 1.00
v:	Sample volume (basic application) [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol x cm]	= 6.3 (at 340 nm)

5.2. Calculation of solid samples

When analyzing solid and semi-solid samples that have to be weighed in for the extraction of the sample, the content is related to the sample weigh-in:

$$\text{Content}_{\text{L-Glutamic acid}}\,[\text{g}/100\ \text{g}] = \frac{C_{\text{L-Glutamic acid}}\,[\text{g/L sample solution}]}{\text{weight}_{\text{Sample}}\ \text{in g/L sample solution}} \times 100$$

Example:

$C_{\text{L-Glutamic acid}}$ = 0.454 g/L weigh-in = 5.02 g/100 mL ≙ 50.2 g/L

$$\text{Content}_{\text{L-Glutamic acid}} = \frac{0.454\ \text{g/L}}{50.2\ \text{g/L}} \times 100 = 0.904\ \text{g}/100\ \text{g (or \%)}$$

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. Recovery of aqueous standard solutions should be within 100 ± 5 %.

For this purpose, we recommend the use of reference materials or standard solutions. For example:

- FAPAS Quality Control Material; Monosodium glutamate (MSG) in corn-based snacks (see validation report for further details)
- Enzytec™ *Liquid* Multi-acid Standard 2 *low* (E8470) with 0.25 g/L L-glutamic acid

6. Performance data

6.1. Specificity & side activities

The glutamate dehydrogenase is specific for L-glutamic acid. No side activities have been identified.

6.2. Interferences

L-ascorbic acid does not interfere at or below 0.5 g/L. In the case of sulfite, no interference was detected at or below 0.01 g/L.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to at least 1250 mg/L L-glutamic acid. The recommended measuring range lies at 10 - 1250 mg/L for a sample volume of 100 µL or 2.5 - 150 mg/L for a sample volume of 1000 µL.

The limit of detection (LoD) was determined according to method DIN 32645:2008-11 in stabilized aqueous solution. This results in an LoD of 4 mg/L L-glutamic acid for a sample volume of 100 µL and 1 mg/L for 1000 µL sample volume.

The limit of quantification (LoQ) was determined by precision profile and confirms a concentration of 10 mg/L and 2.5 mg/L for 100 µL and 1000 µL sample volume, respectively.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital under the following link:

https://eifu.r-biopharm.com/

8. Limits of this method

Test results may vary depending on the sample matrix, the individual test procedure and the laboratory environment. Detection and quantification limits depend on the respective sample matrix and the extraction method. For detailed results and further information, please refer to the current validation report.

For the present enzymatic test, only stated, exemplary matrices could be validated due to the large number of foodstuffs and other sample materials.

When analyzing a non-validated matrix, it is recommended to verify the results obtained by means of spike experiments. If necessary, a validation of the sample matrix of interest will need to be performed.

9. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

10. Disclaimer

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