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 (36 – 46 °F)

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.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test may be used with other foods or samples material, provided that these are subjected to individual validation 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 / 68 – 77 °F) before use. Do not interchange components between kits of different batches

2.3. Storage & stability

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.
Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.
Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).
Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

3.1. General

- Sample preparation for manual and automated testing is the same.
- Bring samples to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with distilled water to a concentration within the measuring range (see performance data).
- For turbid test samples: Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained.
- Degas samples containing carbon dioxide by, for example, stirring them in a beaker or applying a brief ultrasonic pulse (10 s).
- If necessary, decolorize **strongly** colored samples with polyvinyl-polypyrrolidone (PVPP, e.g., 1 g/100 mL sample). Stir or shake for 1 minute and filter or centrifuge at 3000 rpm for at least 5 minutes until a clear supernatant is obtained.
- Clarify samples containing proteins or fat alternatively with Carrez reagents: Weigh an appropriate sample quantity accurately into a 100 mL volumetric flask and add approx. 60 mL distilled water. In case of liquid samples, pipette the sample into a 100 mL volumetric flask or beaker pre-filled with 60 mL distilled water. Add 5 mL Carrez I solution (3.60 g potassium hexacyano-ferrate(II)-trihydrate $K_4[Fe(CN)_6]$ x 3 H_2O/100 mL) and 5 mL Carrez II solution (7.20 g zinc sulfate $ZnSO_4$ x 7 H_2O/100 mL). Mix well after each addition. Adjust the pH with 0.1 M NaOH to a value between 7.5 and 8.5. Transfer into a 100 mL volumetric flask, fill up to the mark, mix and filter using fluted paper filters or syringe filters.
- For fat containing samples, weigh sufficient quantity (considering the measuring range) into a volumetric flask and extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 minutes and filter.
- Crush and homogenize solid and semi-solid samples. Weigh a sufficient quantity of sample in a volumetric flask (considering the measuring range), extract with water; fill up to the mark and filter if necessary (by using fluted paper or syringe filters) or centrifuge in reaction tubes. Use Carrez clarification if necessary.

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 (158 °°F)
- 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 (158 °°F)
- 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 / 68 – 77 °F, 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.

4. Assay procedure

4.1. General assay performance

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

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

4.2. Important notes for assay procedure

- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- Specified incubation times were validated and established at 25 °C (77 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- Use separate tips for each sample and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance
- Always wait for the reaction to end (at least during the first test runs or validation). If the absorbance has not stabilized after the recommended incubation time, continue measuring at 5-minute intervals, for example, until a constant absorbance value is reached.
- If a creep reaction occurs, the reaction will not have finished after stated incubation times and will typically show a constant increase of ΔA. Calculate the analyte-specific ΔA value by plotting the absorbance values against time and performing a linear regression to determine the rate of increase in ΔA per minute related to the creep reaction. Then, extrapolate the absorbance to the time at which reagent 2 is added.
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted 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]	= 6.43 (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 L-Glutamic acid* Validation reports
- Enzytec™ *Liquid L-Glutamic acid* Sample preparation guide
- Enzytec™ *Liquid L-Glutamic acid* Excel templates for results calculation
- Enzytec™ *Liquid L-Glutamic acid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:

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

8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.
For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.
When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.
The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

Upon request, we offer the following services, among others:

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

10. Disclaimer

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