# Enzytec™ *Liquid* Nitrate

**Art. No. E8370**

Version 1 / 2024-02-02



Enzymatic UV assay for the determination of nitrate ($NO_3^-$) 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: meat and fish products, vegetable purees and powders from kale, cabbage, spinach, lettuce, arugula, carrots, and milk/whey powders. 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

Nitrate is reduced to nitrite by nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of the enzyme nitrate reductase (NR):

Nitrate + NADPH + $H^+$ —NR→ nitrite + $NADP^+$ + $H_2O$

The amount of NADPH oxidized in this reaction is stoichiometric to the amount of nitrate. NADPH is measured based on its specific absorption at a wavelength of 340 nm. The result is expressed as mg/L or mg/kg nitrate.

Due to the creep reaction, after the second measurement, a third OD measurement must be carried out after **exactly** 10 minutes. The OD difference will be used to correct the creep reaction.

## 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. However, this depends on the used device.

- Reagent 1: 2 x 50 mL with buffer, NADPH
- Reagent 2: 2 x 12.5 mL with buffer, Nitrate-reductase

### *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

- Sample preparation for manual and automated testing is identical.
- The samples should be brought 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 or centrifuge turbid solutions.
- Degas samples containing carbonic acid.
- Store samples in a cold and dry room protected from light. **Important:** the enzymatic system is highly sensitive for nitrate. Ensure that reagents used for extraction are free from nitrate e.g. water and chemicals.
- A multistepper pipette for adding reagent 1 and reagent 2 is recommended. Use a single tip for each component.
- Use separate tips for each sample extract (and control solutions) to avoid cross-contamination, pre-flush the tip before pipetting.
- Weigh samples with a high fat content into a volumetric flask and extract with hot water; allow sample solution to cool down for fat separation (e.g. 15 min in an ice bath); fill volumetric flask up to the mark with water, filter aqueous solution before testing.
- If necessary, decolorize strongly colored samples with PVPP.
- For clarification of protein-containing samples, Carrez-clarification is recommended.
- Carrez-clarified samples with **low** nitrate concentrations require a Carrez reagent blank (CRB). This has to be prepared by using 15 mL of Carrez-clarified water instead of 15 g of sample. **Important:** perform the pH adjustment with 1 M NaOH. Otherwise, the Carrez-clarified water appears cloudy and the measurement is impaired. This solution will be used **instead** of the water reagent blank (RB) mentioned in section *4. Assay procedure*. Please also note the calculation in section *5. Calculation of results*.

## 4. Assay procedure

Wavelength: 340 nm
Cuvettes: 1.00 cm light path
Temperature: 20 - 37 °C (during the measurement)
Measuring range: 10 - 300 mg/L

| | RB / CRB | Samples / controls |
|---|---|---|
| **Reagent 1**<br>**Sample / control**<br>**Dist. water** | 2000 µL<br>-<br>100 µL | 2000 µL<br>100 µL<br>- |
| Mix, incubate for 3 min at 20 - 37 °C. Read absorbance $\mathbf{A_1}$ at 340 nm, then add: | | |
| **Reagent 2** | 500 µL | 500 µL |
| Mix, incubate for **exactly** 20 min at 20 - 37 °C, read absorbance $\mathbf{A_2}$. | | |
| Incubate, after **exactly** another 10 min read again absorbance $\mathbf{A_3}$. | | |

The (Carrez) reagent blank value must be determined once for each run and subtracted from each sample result.

## 5. Calculation of results

### *5.1. Calculation of sample solutions*

#### *5.1.1. Concentration of nitrate*

$\Delta A_{\text{RB or CRB}} = (A_1 \times df - A_2) - 2 \times (A_2 - A_3)$

$\Delta A_{\text{Sample or control}} = (A_1 \times df - A_2) - 2 \times (A_2 - A_3)$

$\Delta A_{\text{Nitrate}} = \Delta A_{\text{sample or control}} - \Delta A_{\text{RB or CRB}}$

df: Dilution factor
RB: Reagent blank
CRB: Carrez 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 is possibly applicable (max. 1000 µL; refer to validation report). With constant reagent volumes conversion of dilution factor (df) is necessary.
If the sample volume is increased, the test system may be affected. In general, this must be checked depending on the matrix. It is necessary to adjust the (Carrez) reagent blank to the increased sample volume.

$$C_{Nitrate}\ [g/L] = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = 0.2559 \times \Delta A \times F$$

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 [g/mol] | = 62.0 |
| d: | Optical path [cm] | = 1.00 |
| v: | Sample volume (basic application) [mL] | = 0.100 |
| ε: | Extinction coefficient NADHP [L/mmol x cm] | = 6.3 (at 340 nm) |

### *5.2. Calculation of solid samples*

$$Content_{Nitrate}\ [g/100\ g] = \frac{C_{Nitrate}\ [g/L\ sample\ solution]}{weight_{Sample}\ in\ g/L\ sample\ solution} \times 100$$

### *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:

- NIST SRM 3185 Aqueous solution
- NIST 1546a Meat homogenate
- LGC 7114 Kale powder

## 6. Performance data

### *6.1. Specificity & side activities*

The nitrate reductase is specific for nitrate. No side active substances were identified.

### *6.2. Interferences*

Sulfite and sodium chloride do not interfere at or below 7.5 g/L. Neither high citric acid concentration of 10 g/L nor 3 g/L of ascorbic acid interfere in this test. A known interferant for the nitrate reductase is manganese ion (II). Manganese concentrations in food are at maximum 10 mg/kg in oysters and blue mussels. It is certain, that theses concentrations not interfere the nitrate measurement due to the dilution factor after extraction in any way.

### *6.3. Linearity, measuring range & sensitivity*

Linearity is given up to 500 mg/L nitrate, with the recommended measuring range between 10 and 300 mg/L (sample volume of 100 µL).
The limit of detection (LoD) was determined according to method DIN 32645:2008-11 in buffered aqueous solution. This results in an LoD of 7 mg/L and 0.8 mg/L nitrate for a sample volume of 100 µL and 500 µL, respectively. The limit of quantification (LoQ) was determined by precision profile and confirms a concentration of 10 mg/L and 1.5 mg/L for 100 µL and 500 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 suitable sample preparation validation for the sample matrix of interest will need to be performed and validated.

## 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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