

Enzymatic assay for wine, food and feed analysis  
3 x 50 mL R1 + 1 x 16 mL R2 + 1 x 5 mL R3  
(75 assays for manual application, 750 assays on biochemical analyzer)

For in vitro use only  
Store between 2 and 8°C

## Test principle

Primary amino groups are derivatized by o-phthaldialdehyde (OPA) and N-acetylcysteine (NAC) to give isoindoles.

In optimized conditions, isoindoles give a chromogenic complex with a max absorbance at 340 nm, proportional to the concentration of  $\alpha$ -Amino Nitrogen in the sample.

## Assay specifications

- Wavelength: 340 nm (334 - 365 nm)
- Pathlength: 1 cm
- Reading: against air or distilled water
- Temperature: 37 °C
- Method: end-point
- Reaction: 5 minutes
- Sample/reagent: 1/100

## Reagents

- # 1: R1 - Buffer pH > 7.00: 3 bottles with approx. 50 mL
- # 2: R2 - OPA > 5 mM: 1 bottle with approx. 16 mL
- # 3: R3 - CAL: 1 bottle with approx. 5 mL (solution of  $\alpha$ -amino nitrogen = 150 mg/L,  $\text{NaN}_3$  < 0.1%)

Let reagents reach the working temperature before use. This product has been formulated for in vitro diagnostic use. In addition to the possible risk indications, the reagent can contain preservatives (as sodium azide or others), which total concentration is lower than the limits mentioned in Dir. 67/548/CEE e 88/379/CEE and following modifications regarding classification, labelling and packaging of dangerous preparations (Reagents). However, it is recommended to handle the reagents carefully, avoiding ingestion and contact with eyes, mucous membranes and skin; to use reagents according to good laboratory practice. On the material safety data sheet are detailed the operating procedures for the manipulation of this product. Material safety data sheet can be supplied on request.

## Stability

Reagents are ready to use and stable up to the expiry date mentioned on the labels, stored at 2 - 8 °C, if closed and kept in their intact primary container, if not exposed to heat sources and/or pressure variations.

Let the reagent reach the room temperature before use. Close immediately after handling. The reagents must be used correctly, to avoid contamination. R1 reagent is sensitive to oxidation, keep the container open as little as possible. Once opened it is stable 60 days.

## Sample preparation

- Wine can be used directly.
- Use liquid, clear and nearly neutral samples directly or after dilution into the relevant measuring range (see performance data).
- Filter or centrifuge turbid solutions
- Degas samples containing carbon dioxide.
- Crush and homogenize solid samples, weigh out appropriate sample amount and extract with water.

## Procedure

Pipette into cuvettes:	Reagent Blank	Calibrator	Samples
R1 - BUFFER	2000 $\mu$ L	2000 $\mu$ L	2000 $\mu$ L
R3 - Calibrator	---	20 $\mu$ l	---
Sample	---	---	20 $\mu$ L
Dist. water	20 $\mu$ L	---	---
Mix gently and read the absorbances of the standard ( $A1_{\text{standard}}$ ) and of the sample ( $A1_{\text{sample}}$ ). Then add:			
R2 - OPA	200 $\mu$ L	200 $\mu$ L	200 $\mu$ L
Mix gently and wait 5 minutes at 37 °C. Read the absorbances of the standard ( $A2_{\text{standard}}$ ) and of the sample ( $A2_{\text{sample}}$ ).			

## Calculation of results

$$\Delta A = (A2 - df * A1)_{\text{sample or standard}} - (A2 - df * A1)_{\text{reagent blank}}$$

df = dilution factor of the optical densities by reagent volumes:  
 $df = (\text{sample volume} + R1) / (\text{sample volume} + R1 + R2) = 0.910$

Use following general formula to calculate the concentration:

$$\alpha\text{-Amino Nitrogen [mg/L]} = C_{\text{standard [mg/L]}} \times \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}}$$

Since the concentration of the standard is fixed at 150 mg/L, this gives the following calculation formula:

$$\alpha\text{-Amino Nitrogen [mg/L]} = 150 \times (\Delta A_{\text{sample}} / \Delta A_{\text{standard}})$$

## Performance data

1. **Specificity:** this test is specific for  $\alpha$ -Amino Nitrogen. No interferences were detected.
2. **Linearity:** the test is linear up to 200 mg/L. For concentration of  $\alpha$ -amino nitrogen higher than 200 mg/L, dilute the sample with distilled water in the mentioned ranges; repeat the determination and multiply the result by the dilution factor.
3. A proportional variation of the reaction volumes does not change the result.
4. Do not mix reagents from different production lots.
5. Application sheets for automated systems are available on request.
6. Waste disposal: Observe all federal, state and local environmental regulations for waste disposal.

## Disclaimer

The data corresponds to our present state of technology and provides information on our products and their uses. R-Biopharm makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Defective products will be replaced. There is no warranty of merchantability of this product, or of the fitness of the product for any purpose. R-Biopharm shall not be liable for any damages, including special or consequential damage, or expense arising directly or indirectly from the use of this product.