

Colorimetric test for anthocyanin determination in food products
5 x 40 mL R1
(100 assays for manual application, 1000 assays on biochemical analyzer)

For in vitro use only
Store between 15 and 25 °C

Principle

Anthocyanins are the orange-red-purple substances of the wine grapes and then of the wine. These mixtures of anthocyanic polyphenols give chromogens in particular acid conditions and with buffers at particular concentrations. The chromogenic complexes are proportional to the concentration of anthocyanins in the sample, at this wavelength.

This proposed method determines ionized and ionizable anthocyanins; eventual polymerized anthocyanins or complexed with tannic acid or tannins couldn't be determined by this method.

The eventual turbidities of the samples, due to the proteins, are eliminated by the reagent stabilizers.

Assay specifications

- Wavelength: 520 nm or 535 nm
- Pathlength: 1 cm
- Reading: against air or distilled water
- Temperature: 15 - 25 °C
- Method: end-point
- Reaction: 5 minutes
- Sample/reagent: 1/20

Reagents

R1: Buffer (5 x 40 mL); Modified halogenuric buffer, 100 mM, pH < 2; Stabilizers; NaN₃ < 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 and 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

Ready-to-use. Mix gently before use and let the reagent reach the room temperature before use. Close immediately after handling. The reagents have to be used correctly, to avoid contamination.

The reagents are stable up to the expiry date mentioned on the labels, stored at 15 - 25 °C, if closed and kept in their intact primary container, if not exposed to heat sources and/or pressure variations.

Stability after the first opening

The product is stable up to the expiry date mentioned on the labels after the first opening, if stored at 15 - 25 °C.

Sample preparation

- Wine can be used directly.

- Use directly colorless and clear liquid samples if anthocyanins conc. is between 10 - 800 mg/L. Otherwise, dilute with water to reduce it in 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	Sample
Reagent 1	2000 µL	2000 µL
Distilled water	100 µL	-
Sample	-	100 µL

Mix carefully. Incubate for 5 minutes at room temperature; read the absorbance of sample and reagent blank.

Calculation of results

Use this general formula to calculate the concentration:

At 520 nm

$$\text{Anthocyanins (mg/L)} = (A_{\text{sample}} - A_{\text{reagent blank}}) \times 420$$

At 535 nm

$$\text{Anthocyanins (mg/L)} = (A_{\text{sample}} - A_{\text{reagent blank}}) \times 470$$

Performance data

1. The test is specific for anthocyanins. No interferences were detected.
2. Linearity: The test is linear up to 800 mg/L. For concentrations of anthocyanins higher than 800 mg/L, pre-dilute the sample with distilled water in the mentioned range and multiply the result by the dilution factor.
3. Applications on routine analyzers may be totally different from what developed as manual determination; in addition, the procedures are specific for each analyzer. Application sheets for automated systems are available on request.
4. A lot of factors, as ambient temperature, the working reagent temperature, wash accuracy and the type of spectrophotometer may affect the tests performances.
5. Do not mix reagents from different production lots.
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.