

Total polyphenols quantitative colorimetric determination in wine, food and beverages
 2 x 80 mL R1 + 1 x 80 mL R2 + 2 x 40 mL R3 + 1 x 5 mL R4
 (80 assays for manual application, 800 assays on biochemical analyzer)

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

Principle

Polyphenols react in basic environment with Folin reagent optimized and modified. The chromogenic complex is proportional to the concentration of polyphenols in the sample, at this wavelength.

Assay specifications

- Wavelength: 700 nm (620 - 760 nm)
- Pathlength: 1 cm
- Reading: against air or distilled water
- Temperature: 37 °C
- Method: end-point
- Reaction: 15 minutes
- Sample/reagent: 1/100/50 (for red wines)
1/20/10 (for white wines)

Reagents

1: Folin reagent modified: 2 bottles of 80 mL

2: Sodium carbonate >10 g/L: 1 bottle of 80 mL

3: Sodium hydroxide >1 g/L: 2 bottles of 40 mL

4: Standard liquid: Solution of gallic acid 3 g/L,
NaN₃ < 0.1%, 1 bottle of 5 mL

All reagents are 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.

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.

Waste disposal: Observe all federal, state and local environmental regulations for waste disposal.

Stability

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. In case of damaging of the primary container organize the waste disposal.

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. In case

of microbial contamination the preparations must be eliminated.

Sample preparation

- Wine can be analyzed directly.
- For samples of red wine with a very intense color, prepare a 1:3 (1+2) predilution of the sample with distilled water; repeat the determination as for red wines and multiply the result by the dilution factor (X3).
- Use clear samples directly if the concentration of total polyphenols (such as gallic acid) is in the range of 0.020 - 3 g/L; otherwise, dilute with water to bring the concentration into this range (see performance data).
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- For fatty samples ask for use and for possible treatment.
- For solid or semi-solid samples, ask for specific pre-treatment procedures and for calculation of results.

Procedure for RED WINES

Use R4 - Standard liquid as it is, without dilution.

Pipette into cuvettes:	Standard	Sample	Reagent Blank
Reagent 1	2000 µL	2000 µL	2000 µL
Sample	-	20 µL	-
Reagent 4 (Standard)	20 µL	-	-
Distilled water	-	-	20 µL
Mix and incubate for about 60 seconds at room temperature, read the absorbance of Standard (ST), Sample (S) and Reagent Blank (RB). Then add:			
Reagent 2 (Sodium carbonate)	1000 µL	1000 µL	1000 µL
Mix carefully. Incubate for 15 minutes at room temperature; read the absorbance of Standard (ST), Sample (S) and Reagent Blank (RB).			

Procedure for WHITE WINES

Dilution of the Standard:

Dilute R4 - Standard liquid 1:5 (1+4) with distilled water, resulting in a value of 0.6 g/L, to be used only for white wines.

Pipette into cuvettes:	Standard	Sample	Reagent Blank
Reagent 1	2000 µL	2000 µL	2000 µL
Sample	-	100 µL	
Reagent 4 (Standard)	100 µL	-	-
Distilled water			100 µL
Mix gently each cuvette and, after about 60 seconds, read the absorbance of Standard (ST), Sample (S) and Reagent Blank (RB). Then add:			
Reagent 2 (Sodium carbonate)	1000 µL	1000 µL	1000 µL
Mix gently and wait 15 minutes. Read the absorbance of Standard (ST), Sample (S) and Reagent Blank (RB).			

Disclaimer

The data corresponds to our current state of technology and provides information about our products and their use. R-Biopharm does not provide any warranty, express or implied, other than that relating to the standard quality of the materials of which its products are made. In the event that these materials are found to be defective, R-Biopharm undertakes to provide replacement products. There is no warranty of merchantability or fitness of the product for a particular purpose. R-Biopharm shall not be held liable for damages, including special or indirect damages, or expenses arising directly or indirectly from the use of the product.

Calculation of results

Use this general formula to calculate the concentration:

$$\Delta A = (A_2 - df \times A_1)_{\text{sample or standard}} - (A_2 - df \times A_1)_{\text{RB}}$$

$$df = (\text{sample} + R_1) / (\text{sample} + R_1 + R_2)$$

$$df_{\text{RED WINES}} = 0.669$$

$$df_{\text{WHITE WINES}} = 0.677$$

$$C_{\text{sample}} [\text{mg/L}] = \frac{\Delta \text{Abs}_S - \Delta \text{Abs}_{\text{RB}}}{\text{Abs}_{\text{ST}} - \text{Abs}_{\text{RB}}} \times C_{\text{ST}} [\text{mg/L}]$$

Since the concentration of the standard is set at 3 g/L of gallic acid for red wines and 0.6 g/L for white wines, the calculation becomes as following:

$$\text{Red wines: } C_{\text{sample}} [\text{g/L}] = 3 \times (\Delta A_{\text{sample}} / \Delta A_{\text{standard}})$$

$$\text{White wines: } C_{\text{sample}} [\text{g/L}] = 0.6 \times (\Delta A_{\text{sample}} / \Delta A_{\text{standard}})$$

Performance data

- Specificity:** This test is specific to total polyphenols. No interference is known.
- Linearity:** For concentration of total polyphenols higher than 3 g/L, dilute the sample with distilled water in the mentioned measuring range.
- The volumes can be varied proportionally.
- Applications on automatic chemistry analyzers are available on request.
- The result can also be expressed as follows:
 - TANNIC ACID = 1.57 x Total polyphenols (as Ac. Gallic)
 - CATECHINS = 1.24 x Total polyphenols (as Ac. Gallic).