



**KB03015**

# **Anthocyanins Assay Kit**

**96 well plate  
100/200/400 tests**

**Votre interlocuteur en France, Belgique, Luxembourg et Suisse :  
LIBIOS**

**83, rue Edmond Michelet - 69490 Pontcharra Sur Turdine - France  
Tél. : +33 (0)4 74 13 03 02 - Fax : +33 (0)4 74 05 28 25 –  
Mail : [info@libios.fr](mailto:info@libios.fr) - [www.libios.fr](http://www.libios.fr)**

## Table of contents

1.	General information	1
2.	Technical specifications	2
3.	Materials and storage	3
4.	Introduction	4
5.	Assay principle	5
6.	Assay preparation	6
7.	Sample preparation	7
8.	Assay protocol	8
9.	Data analysis	9
10.	Troubleshooting	10
11.	Additional information	12
12.	Related products	12
13.	Warranties and limitation of liability	13

## 1. General information

### PRECAUTIONS

Please read this manual carefully before beginning the assay.

This product is designed for **research use only**. It is not approved for human or animal use or clinical diagnosis. All chemicals should be handled with care and in accordance with laboratory safety practices. It is recommended to use basic Personal Protective Equipment.

Do not use after the expiration date stated on the packaging.

Do not mix or substitute reagents or materials from other kit batches or vendors.

For the **material safety data sheet** (MSDS) please contact us at [info@bioquochem.com](mailto:info@bioquochem.com)

### TECHNICAL RECOMMENDATIONS

Store reagents as indicated in **Materials and storage** section.

Be sure to keep the bottle capped when not in use.

Let the components reach room temperature (RT) before use.

Immediately before use, gently invert and rotate reagent bottles several times to mix the contents thoroughly.

Avoid foaming or bubbles when mixing or reconstituting components.

Avoid cross contamination of samples or reagents by changing pipette tips between sample, standard and reagent additions.

Be sure to use the optimal microplate for the assay. Flat bottom transparent microplates for UV/VIS applications, and black microplates for fluorescence measurements.

## 2. Technical specifications

### Available sizes

100/200/400 tests

### Required sample volume

20 µL/test

### Compatible samples

Foods, beverages and plant samples

### Type of detection

Colorimetric (510 and 700 nm)

### 3. Materials and storage

#### MATERIALS SUPPLIED

Item	No. Tests	Units	Storage
Reagent A	100	1	RT
	200	1	
	400	2	
Reagent B	100	1	RT
	200	1	
	400	2	

#### MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (ddH<sub>2</sub>O) as Milli-Q Ultrapure Water
- Labware materials (micropipettes, tubes, stirring/mixing equipment)
- Transparent 96-Well Microplate
- Colorimetric microplate reader – equipped with filter for OD 510/700 nm

#### STORAGE CONDITIONS

On receipt, store kit components as indicated above. Under these conditions, the reagents are stable in the original packaging until the expiration date stated on the outside of the box.

## 4. Introduction

Anthocyanins represent the most important group of water-soluble pigments detectable by the human eye in the plant kingdom. These pigments are responsible for the blue, purple, and red color of many plant tissues. Anthocyanins have important functions in plant physiology as well as possible health effects thanks to their antioxidants, anti-inflammatory, and antimicrobial properties.

Anthocyanins are phenolic compounds belonging to the family of flavonoids. Anthocyanins are the glycosylated forms of anthocyanidins, which are polyhydroxy (-OH) or polymethoxy (-OCH<sub>3</sub>) derivatives of the flavylum cation (2-phenylbenzopyrilium ion). Glycosylation (attachment of sugar moieties) of anthocyanidins can occur at different positions of the molecule with the 3 positions being dominant. The glycosylation of flavonoids increases their solubility and stability in plants.

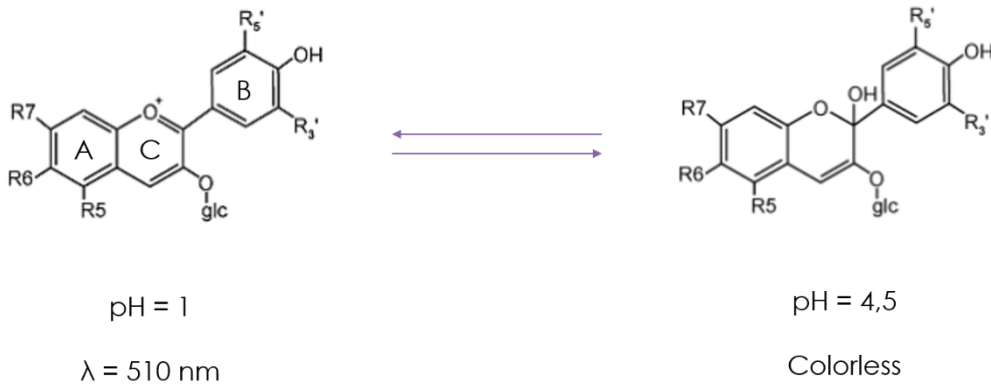
The antioxidant activity of anthocyanins depends on their chemical structure: number and position of the hydroxyl groups and the conjugated double bonds, as well as on the presence of electron donors in the structural ring.

**BQC Anthocyanins Assay Kit is an easy, fast, and reliable method for detecting anthocyanins in food, beverages, and plant samples.**

## 5. Assay principle

Anthocyanin pigmentation is largely due to the positive charge on the C ring of the molecule, but this charge is pH dependent. At a pH of 1.0, the C ring is positively charged, and the molecule is pigmented. At pH values 4.5 and higher the positive charge is neutralized, and anthocyanin molecules become colorless. At a pH of 1.0 anthocyanins strongly absorb light between 460 and 550 nm with an absorption maximum at about 510 nm.

The BQC Anthocyanins Assay Kit determine the anthocyanin concentration by measuring the absorbance at 510 nm after sample acidification. Turbidity (haze) is corrected by measuring the absorbance at 700 nm.



*Principle of Anthocyanins Assay Kit*


## 6. Assay preparation

### REAGENT PREPARATION

All assay reagents are ready to use as supplied.

### PLATE SET UP

**BQC recommends running the samples and blanks at least for duplicate (triplicate recommended).** There is no specific pattern for using the wells on the plate. A proposed layout of samples (S), and sample blanks (SB) to be measured in duplicate is shown below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S5	S5	S9	S9	S13	S13	S17	S17	S21	S21
B	SB1	SB1	SB5	SB5	SB9	SB9	SB13	SB13	SB17	SB17	SB21	SB21
C	S2	S2	S6	S6	S10	S10	S14	S14	S18	S18	S22	S22
D	SB2	SB2	SB6	SB6	SB10	SB10	SB14	SB14	SB18	SB18	SB22	SB22
E	S3	S3	S7	S7	S11	S11	S15	S15	S19	S19	S23	S23
F	SB3	SB3	SB7	SB7	SB11	SB11	SB15	SB15	SB19	SB19	SB23	SB23
G	S4	S4	S8	S8	S12	S12	S16	S16	S20	S20	S24	S24
H	SB4	SB4	SB8	SB8	SB12	SB12	SB16	SB16	SB20	SB20	SB24	SB24

*Example of plate layout for the Anthocyanins Assay Kit*



## 7. Sample preparation

**The following sample preparation protocols are intended as a guide only. The optimal conditions for sample preparation must be determined by the end user.** It is recommended to use fresh samples. If it is not possible, aliquot and store samples appropriately with minimal freeze/thawing.

**Anthocyanins Assay Kit can be used to determine the anthocyanin content in a wide variety of samples like foods, beverages and plant extracts.**

**Food and beverages.** Fruit juices and other beverages such as wine, tea, and coffee can be directly measured with appropriate dilutions. If it is required, clarify the sample through filtration prior performing the assay. Ensure that the selected filter is appropriate for filtering your samples, avoiding polyphenols retention.

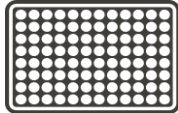
For the analysis of other samples like **fruits, vegetables, and plants** an extraction step is usually required. The extraction method varies based upon the sample type. The most common extraction solvents include acid/methanol, acid/ethanol, or acetone.

Reagents and materials required for sample preparation are not supplied with the kit. Before doing sample preparation, consider the volume of sample required per test; see **Technical specifications** section.

## 8. Assay protocol

Prepare and mix all reagents thoroughly before use. Each sample and sample blank should be assayed at least in duplicate.

1



Set up the plate design

2



Add **200 µL** of **Reagent A** in **sample** wells.

**If the absorbance is  $\geq 1.4$  (510 nm) after the addition of Reagent A samples should be diluted**

3



Add **200 µL** of **Reagent B** in **sample blank** wells

4



Add **20 µL** of **sample** in all wells

5



Let the reaction run for **10 minutes** at **RT**

6



Read the **absorbance** of all wells in end point mode at **RT** at **510 nm (anthocyanin maximum absorption)** and at **700 nm** (for turbidity correction)

If you need to **adapt this kit** for another form of the assay (for example cuvette), **contact us at [info@bioquochem.com](mailto:info@bioquochem.com)**

## 9. Data analysis

### ANALYSIS OF THE SAMPLES

- Calculate the **absorbance (A) due to anthocyanins** present in the sample using the following formula:

$$A (\text{Anthocyanins}) = (A_{S510\text{nm}} - A_{S700\text{nm}}) - (A_{SB510\text{nm}} - A_{SB700\text{nm}})$$

Where  $A_S$  is the absorbance of sample wells and  $A_{SB}$  is the absorbance of sample blanks.

- Calculate the anthocyanin concentration of the sample expressed as **cyanidin 3-glucoside equivalents** (mg/L) as follows:

$$\text{Anthocyanins (mg/L)} = \frac{A (\text{Anthocyanins}) \cdot \text{DF} \cdot \text{MW} \cdot 10^3}{\epsilon \cdot l}$$

Where **A (Anthocyanins)** is the absorbance previously calculated, **DF** is the dilution factor; **MW** is the molecular weight for cyanidin-3-glucoside (449.2 g/mol); **10<sup>3</sup>** is a factor for conversion from g to mg, **ε** is the molar extinction coefficient for cyanidin-3-glucoside (26900 L · mol<sup>-1</sup> cm<sup>-1</sup>) and **l** is the pathlength in cm (0.6 cm for a 96-well microplate).

## 10. Troubleshooting

This troubleshooting table provides potential sources and solutions for common problems observed with BQC Assay Kits. **The problems listed below could occur when using any BQC Assay Kit.** They are not specific for this assay kit.

Problem	Possible Cause	Recommended Solution
Wells have color but there is no reading	Plate read at incorrect wavelength	Check the wavelength used in the assay
	Incorrect microplate	Use the correct microplate for your application UV/Vis: transparent Fluorescence: black wells/transparent bottom
Standard readings do not follow a linear pattern	Pipetting errors in preparation of standards	Avoid pipetting small volumes (<5 $\mu$ L) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique
	Standard stock is at incorrect concentration	Always refer to dilutions described in <b>Assay preparation</b>
	Improperly thawed reagents	Thaw all components completely and mix well before use
	Use of improperly stored reagents	Store the components appropriately Use fresh components from the standard curve
	Incorrect incubation times or temperatures	Refer to <b>Assay protocol</b>
Dispersion of standard and sample readings	Pipetting errors	Avoid pipetting small volumes (<5 $\mu$ L) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique

Problem	Possible Cause	Recommended Solution
Sample erratic values	Samples contain interfering substances	Dilute sample further (if possible)
	Inappropriately stored samples or samples used after multiple freeze-thaw cycles	Use fresh samples or store appropriately until use
	Samples not deproteinized	Use an appropriate deproteinization protocol
	Cells/Tissue samples not homogenized completely	Repeat the sample homogenization
	Inappropriate sample dilution buffer	Refer to <b>Assay preparation</b>
Sample reading fall outside the detection range	Samples are too diluted/concentrated No analyte/activity is observed in the sample	Re-assay using different sample dilutions

## STILL HAVING PROBLEMS?

Contact BQC if you have any further questions, our team will be pleased to help you:



Phone

+ 34 985 26 92 92



E-mail

[info@bioquochem.com](mailto:info@bioquochem.com)



Business hours

Monday-Thursday: 8.30 to 17.00 (CEST)  
Friday: 8.00 to 15.00 (CEST)

## 11. Additional information

**BQC Anthocyanins Assay Kit** is a quick (< 15 minutes) assay for determining anthocyanins in a wide variety of samples.

The use of acidified solvents to extract anthocyanins from sample can interfere with the assay (overestimation of results).

If unexpected results are obtained running your samples, please contact us at [info@bioquochem.com](mailto:info@bioquochem.com)

## 12. Related products

More products available on [bioquochem.com](http://bioquochem.com)

Reference	Product
KB03006	Polyphenols Quantification Assay Kit
KF01001	DMPD Antioxidant Capacity Assay Kit
KB03007	Thiol Quantification Assay Kit

## 13. Warranties and limitation of liability

BQC shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if BQC has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, downtime, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by BQC's gross negligence. Any and all liability of BQC hereunder shall be limited to the amounts paid by the buyer for the product.

Buyer's exclusive remedy and BQC's sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to BQC within 30 days of shipment.

**Expiration date:** 1 year from the date of fabrication. Expiration date is indicated on the outside of the box.

For further details, please refer to our website [bioquochem.com](http://bioquochem.com)



Votre interlocuteur en France, Belgique, Luxembourg et Suisse :  
**LIBIOS**

83, rue Edmond Michelet - 69490 Pontcharra Sur Turdine - France  
Tél. : +33 (0)4 74 13 03 02 - Fax : +33 (0)4 74 05 28 25 –  
Mail : [info@libios.fr](mailto:info@libios.fr) - [www.libios.fr](http://www.libios.fr)