



Dynamic Test Kits for R&D  
and Quality Control

# Proanthocyanidins (PAC) Assay kit

*DMAC Method*

*KB-03-017*

*100/200/500 test*



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➤ This kit is for R&D use only

All these chemicals should be handled with care

# Introduction

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Proanthocyanidins also named as PACs, are polymeric condensation products (condensed tannins), responsible for many bitter and astringent flavors in food products such as wine, chocolate, beer, and cranberries. They are widely distributed in nature and represent the most abundant flavonoids consumed in the diet. Recent attention has been given to these compounds because of their health-promoting properties toward chronic diseases and are considered functional ingredients in many foods and beverages because of their positive effects on human health.

# Materials

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Bioquochem Proanthocyanidins Assay kit KB-03-017 for 100 test contains:

Product	Quantity	Storage
Reagent A	1 bottle of 30 ml	RT
Reagent B	1 vial of 2 ml	RT
Reagent C	2 vials of 2 ml	RT
Standard	1 vial 2 ml	4°C

Bioquochem Proanthocyanidins Assay kit KB-03-017 for 200 test contains:

Product	Quantity	Storage
Reagent A	1 bottle of 60 ml	RT
Reagent B	1 vial of 5 ml	RT
Reagent C	4 vials of 2 ml	RT
Standard	1 vial 2 ml	4°C

Bioquochem Proanthocyanidins Assay kit KB-03-017 for 500 test contains:

Product	Quantity	Storage
Reagent A	2 bottles of 125 ml	RT
Reagent B	1 vial of 5 ml	RT
Reagent C	5 vials of 2 ml	RT
Standard	1 vial 2 ml	4°C

# Assay principle

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The 4-dimethylaminocinnamaldehyde (DMAC) reacts with procyanidins' A cycle's C8, in terminal units of proanthocyanidin. This reaction produces a green/blue colored compound that can be measured by spectrophotometry.

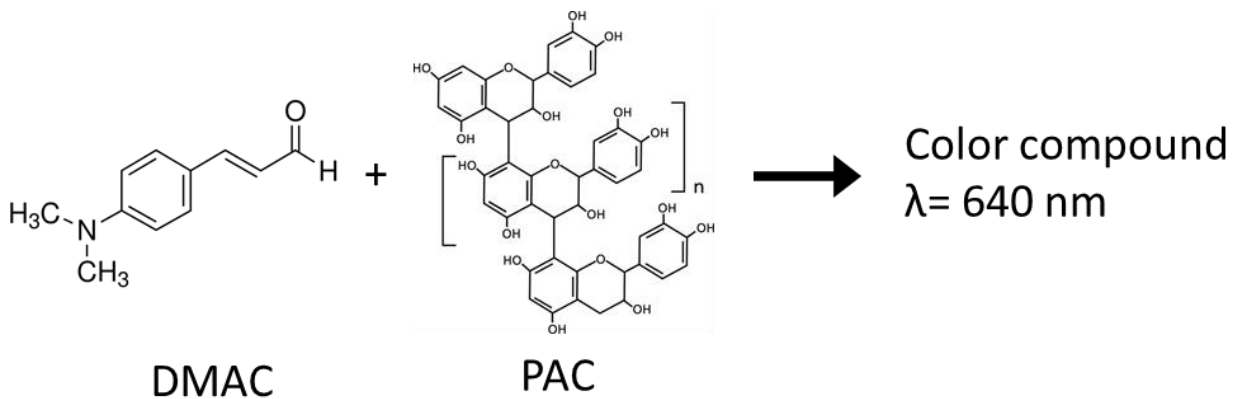


Figure 1. Principle of the assay reaction

# Reagent preparation

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## Reagent C:

Add 500  $\mu\text{l}$  of Reagent B in one vial of Reagent C. Mix gently and store at 4°C.

## Standard solutions:

KB03017-100 and KB03017-200: Prepare standard reagent by adding exactly 1ml of Reagent A to the standard tube.

KB03017-500: Prepare standard reagent by adding exactly 1 ml of Reagent A to the standard tube and then dilute it 1:10 to perform the assay.

Prepare calibration curves in tubes as shown in Table 1.

Table 1. Reagent volumes needed to carry out the standard curve.

Standard ( $\mu\text{L}$ )	Reagent A ( $\mu\text{L}$ )	[Standard] $\mu\text{g/ml}$
0	200	0
2.5	197.5	25
5	195	50
10	190	100
15	185	150
20	180	200

# Protocol

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## Performing the assay

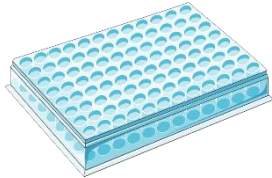
1. Prepare the DMAC reagent. Add 500  $\mu\text{l}$  of Reagent B in one vial of Reagent C. Once mixed store at 4°C.
2. Considering a 96 well plate, add 230  $\mu\text{l}$  of Reagent A, 10  $\mu\text{l}$  of sample or standard and 10  $\mu\text{l}$  of DMAC reagent in each well. Do it for each sample that is going to be measured.
3. Leave the samples in agitation in minimal light conditions for 15 min.
4. Measure the absorbance at 640 nm.

# Assay Protocol

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## Short protocol:

1



Prepare all reagents and 96 well plate.

2



Add 230  $\mu$ l of Reagent A solution.

3



Add 10  $\mu$ l of reagent C previously prepared and 10  $\mu$ l of sample or standard.

4



Let the reaction run, shaking plate, without light for 15 minutes.

5



Read the absorbance at 640 nm.



# Data analysis

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1. Plot the absorbance at 640nm of standards as function of their final concentrations (Table 1). See Figure 2 for a typical standard curve.

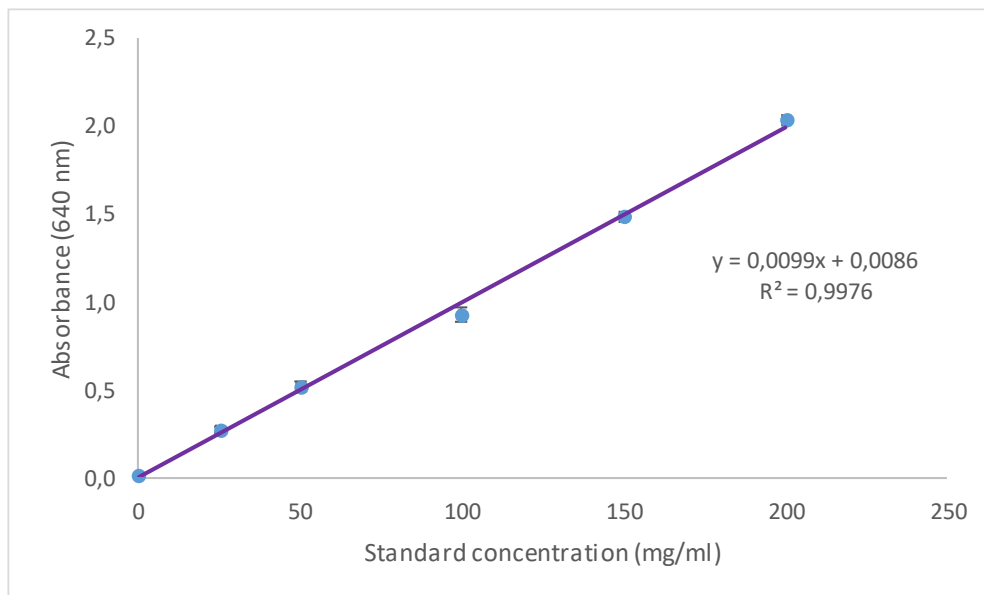


Figure 2. Example of the standard representation

2. Calculate the PAC concentration of the samples using the equation obtained from the linear regression of the standard curve replacing the  $A_{640\text{nm}}$  values for each sample.

# Warranties and Limitation of Liability

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Our partner Bioquochem 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 Bioquochem has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, down time, 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 Bioquochem's gross negligence. Any and all liability of Bioquochem hereunder shall be limited to the amounts paid by buyer for product.

Buyer's exclusive remedy and Bioquochem'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 Bioquochem within 30 days after arrival of the material at its destination.

Expiration date: 1 year from the date of delivery

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