ABEL® ANTIOXIDANT TEST KIT
for SUPEROXIDE and other FREE RADICALS
with PHOLASIN®

ABEL-21

A chemiluminescent test for measuring the capacity of a liquid sample such as water, plasma, serum, synovial fluid, etc., as well as dissolved extracts, to scavenge free radicals such as the superoxide anion

CONTENTS

Kit components sufficient for 25 tests

1 x bottle Pholasin® (reconstitute to 5mL)

1 x 50mL Reconstitution and Assay Buffer

1 x 25mL Solution A
1 x 10mL Solution B

2 x bottles L-Ascorbic acid (reconstitute to 5mL)

REAGENT	FORMAT	TEMPERATURE	SHELF LIFE
PHOLASIN® (Antioxidant)	Freeze Dried	-20ºC	up to 12 months
	Reconstituted	DISCARD UNUSED PRODUCT	
RECONSTITUTION & ASSAY BUFFER FOR PHOLASIN®	Liquid	2-8ºC	up to 1 month
	Frozen	-20ºC or lower	up to 12 months
SOLUTION A	Liquid	Room Temp.	up to 12 months
SOLUTION B	Liquid	2-8ºC	up to 12 months
L-ASCORBIC ACID (reconstitute to 5mL)	Freeze Dried	-20ºC or lower	up to 12 months
	Reconstituted	DISCARD UNUSED PRODUCT	

If any packs are damaged or bottles appear to have leaked, do not use the items, but contact your supplier for advice.

This kit is supplied for research use only. Responsibility will not be accepted for misuse of the kit components. This kit contains glass items, and the use of sharps is recommended; these should be handled with due care and disposed of correctly according to good laboratory practice.

All reagents must be at room temperature (or above) at the start of the assay. Crystals may form in Solution A during storage at 2-8°C but they will dissolve easily with gentle warming.

Pholasin® and L-Ascorbic acid are supplied in vials that have been sealed under vacuum. **IT IS IMPORTANT THAT YOU DO NOT REMOVE THE RUBBER INSERT UNTIL THE BOTTLES HAVE BEEN RECONSTITUTED WITH BUFFER THAT HAS BEEN INJECTED THROUGH THE SEPTUM.**

The kit contains sufficient reagents for 25 tests of 1 mL each.

INTRODUCTION

This is an excellent assay for measuring the antioxidant activity of materials that quench superoxide such as ascorbic acid. Many natural plant extracts quench superoxide in a similar way making this a very easy and reliable test to use with natural materials used in supplements and cosmetics.

The ABEL®-60 series is to be used for measuring superoxide dismutase (SOD) activity.

Superoxide is generated in a cuvette containing Pholasin® with and without a 50µL sample of liquid containing a material to be tested with unknown antioxidant capacity. Ingredients should be dissolved in buffers or water. For blood samples serum or plasma from blood collected in heparin or citrate can be used. EDTA **plasma cannot be used.** Synovial fluid, cell supernatants, process water etc can also be used.

Superoxide is generated in the assay instantaneously when Solution B is injected into a cuvette containing Solution A. If Pholasin® is present when the superoxide is generated light will be emitted. If there are antioxidants (other than Pholasin®) in the sample capable of scavenging superoxide then these will compete with the Pholasin® for the superoxide and less light will be detected. The antioxidant capacity of the sample is expressed as the percentage reduction in peak light emission compared to controls without sample. The reduction can also be expressed in equivalent ascorbate units, to obtain these, an ascorbate standard curve is run as part of the assay (see section Standard curve for ascorbate).

This kit can also be used to generate an ABEL-RAC mg score. Contact Knight Scientific for more information.

EQUIPMENT REQUIRED

A tube luminometer with sample mixer, temperature control (25°C or ambient) and automatic injectors is ideal. However, if your luminometer does not mix then it is suggested that the cuvette should be mixed with either a vortex mixer or by hand immediately before placing in the luminometer.

PROTOCOL

Reconstitution of Pholasin®

The Pholasin® has been specially formulated to be reconstituted with 5mL of Reconstitution and Assay Buffer.

1. Load a syringe with 5mL Reconstitution and Assay Buffer.

2. Fit a needle (1 inch, 21 gauge; 0.8 x 25mm) to the syringe.

3. Remove the protective screw cap from the vial of Pholasin® making sure to leave the rubber insert in place. Carefully push the needle through the rubber septum. There is no need to push the plunger because as soon as the septum is pierced, the syringe contents will be drawn automatically into the vial. However, be sure that the syringe is emptied completely.

4. Replace the screw cap and invert and roll the bottle at least 5 times to dissolve the contents.

5. Reconstituted antioxidant Pholasin® **MUST NOT BE FROZEN** and should be stored at 2-8°C for up to 1 day. Any leftover reconstituted Pholasin® must be discarded.

TEST PROCEDURE

Pipette into a luminometer cuvette:

- 150µL assay buffer or 100µL assay buffer + 50µL sample
- 200µL Pholasin®
- 550µL Solution A

Mix contents of cuvettes with a vortex mixer, or by hand, before placing them in the luminometer, (set at 25°C or ambient temperature). Start the assay ensuring that the mixer is on.

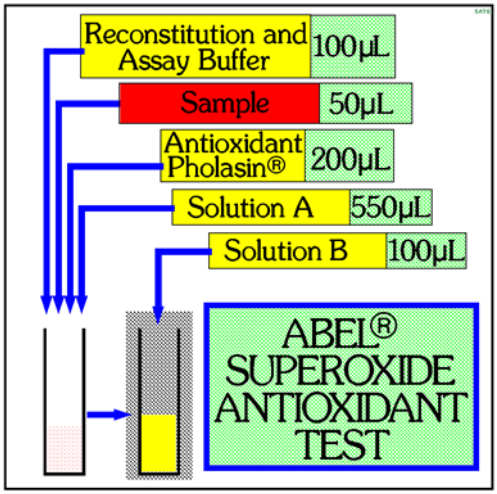
While the cuvette is in front of the light detector, inject 100µL Solution B. The peak is reached within 5 seconds. Record the light of emission for a further 20seconds. A control, without sample, but with a volume of buffer equal to the volume of the sample is run with each assay.

The antioxidant capacity of the sample can be expressed as the percentage reduction of peak luminescence as follows:

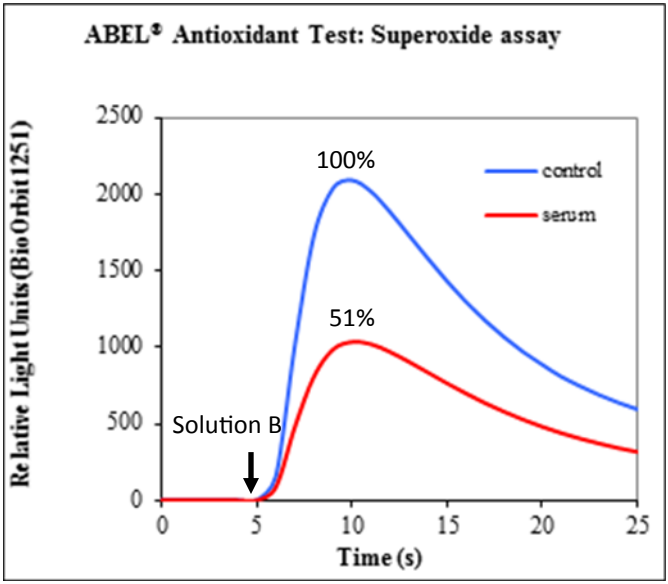
$$[(\text{peak, control}) - (\text{peak, sample})] \times 100/(\text{peak, control})$$

The reduction of peak luminescence can also be expressed in equivalent ascorbate units. To obtain equivalent ascorbate units, an ascorbate standard curve is run as part of the assay (see section Standard curve for ascorbate).

SUMMARY



TYPICAL ANALYSIS



STANDARD CURVE FOR ASCORBATE

Reconstitution of L-Ascorbic acid (sodium salt)

1. The L-Ascorbic acid has been specially formulated to be reconstituted with 5mL of Reconstitution and Assay Buffer to give a final concentration of 1mmol L^{-1}. Keep reconstituted bottle in the dark when not in use as the product is light sensitive.

   Load a syringe with 5mL Reconstitution and Assay Buffer.

   Fit a needle (1 inch, 21 gauge) to the syringe.

   Remove the protective screw cap from the vial of L-Ascorbic push the needle through the rubber septum. There is no need to push the plunger because as soon as the septum is pierced, the syringe contents will be drawn automatically into the vial.

   Replace the screw cap and invert and roll the bottle at least 5 times to dissolve the contents.

   Any unused ascorbate should be discarded.

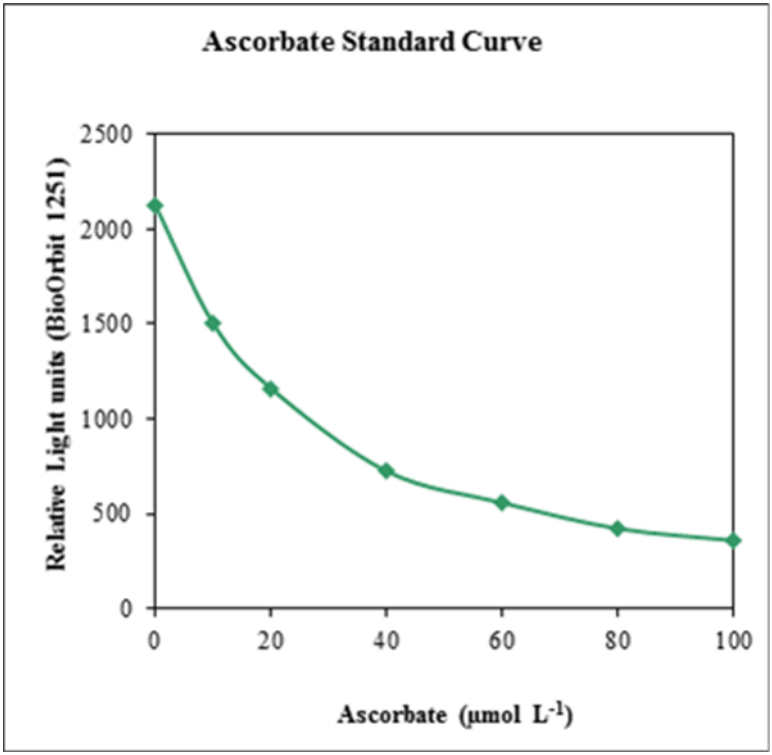
2. Pipette into a cuvette: 200µL Pholasin
   50µL R&A Buffer
   550µL Solution A
   100µL of 1mmol L^{-1} ascorbate + R&A buffer, as shown in the table below.

3. Inject 100µL Solution B when the cuvette is in the light measuring position.

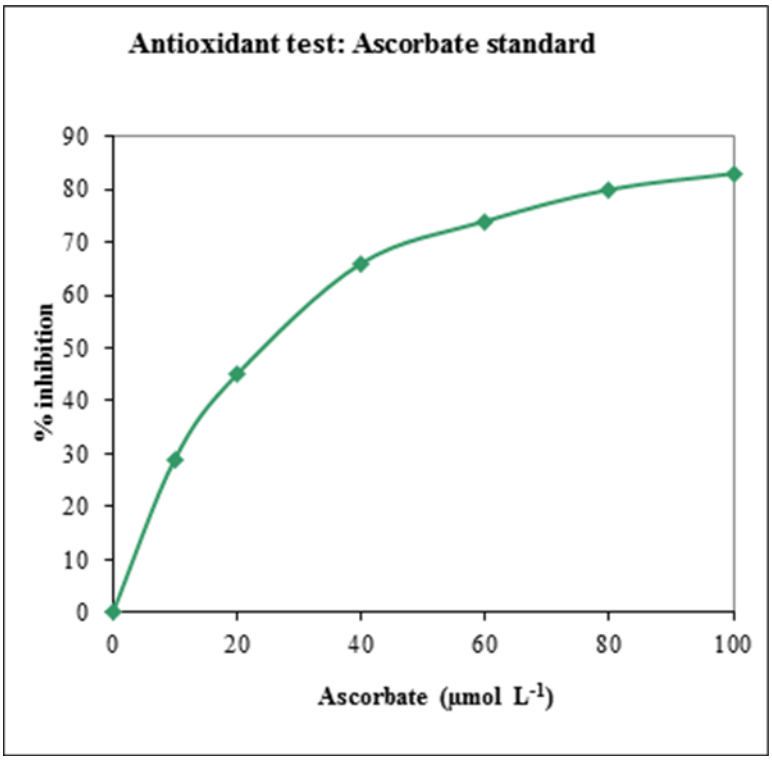
4. The total volume in the cuvette is 1mL.

Volumes of ascorbate and R&A Buffer added to cuvette.

1mmol L^{-1} ascorbate (µL added)	R&A Buffer (µL added)	Ascorbate (µmol L^{-1}) in 1mL	Ascorbate (mmol L^{-1}) in 50µL sample
0	100	0	0
10	90	10	0.2
20	80	20	0.4
40	60	40	0.6
60	40	60	0.8
80	20	80	1.6
100	0	100	2.0



Ascorbate standard curve: BioOrbit 1251 tube luminometer



Results can be expressed as percent inhibition compared to control or as ascorbate equivalent antioxidant units.

Ascorbate Equivalent Antioxidant Units:

51% inhibition in 50µL sample in a 1:20 dilution ≡ 25µmol L^{-1}

→ 500 µmol L^{-1} ascorbate equivalent units in pure sample

For further help and advice, please telephone, fax or e-mail:

Knight Scientific Limited

15 Wolseley Close Business Park

Plymouth, PL2 3BY, UK

telephone: +44 (0) 1752 565 676

e-mail: Info@knightscientific.com

www.knightscientific.com