ABEL® ANTIOXIDANT TEST KIT

for SUPEROXIDE and other FREE RADICALS

with PHOLASIN®

ABEL-21M2

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

Microplate luminometer kit sufficient for 100 x 200 µL tests

1 x bottle of Pholasin® (reconstitute to 5mL)

1 x bottle of Reconstitution-and-Assay (R-&-A) Buffer (25 mL)

1 x bottle of Solution A (25 mL)

1 x bottle of Solution B (10 mL)

2 x bottles of L-ascorbic acid sodium salt (freeze-dried,

reconstitute with 5 mL of 2.)

1 x 96 well white microplate

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	

Store the kit at -20°C until use. After use, store individual components according to the instructions below. If any packs are damaged or bottles appear to have leaked do not use the items but contact your supplier for advice.

MATERIALS REQUIRED BUT NOT PROVIDED

A microplate luminometer with at least one automatic reagent dispenser and, preferably, temperature control (25°C or ambient).

Syringes (5 mL) and needles (1 inch, 21 gauge) for reconstitution of freeze dried reagents.

Precision pipettes with a range of 10 µL to 1000 µL

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.

The ABEL-21M2 antioxidant assay using superoxide provides a rapid method for the assessment of the antioxidant capacity of a range of samples including foods and nutraceuticals, beverages and cosmetic ingredients and products. It is especially suitable for measuring the antioxidant capacity of antioxidants such as ascorbic acid that are chemical quenchers of superoxide.

ASSAY PRINCIPLE

Superoxide at high concentration is immediately generated in the assay when Solution B is injected into a microplate well containing Solution A. If Pholasin® is present when the superoxide is generated light will be emitted. If there are antioxidants in a sample capable of scavenging superoxide then these will compete with the Pholasin® for the superoxide and less light will be emitted. The antioxidant capacity of the sample may be expressed in 3 ways:

Ascorbic acid equivalent units, by comparison to a standard curve using the ascorbic acid standard supplied.

The percentage reduction in peak light emission compared to a no-sample control (% inhibition).

ABEL® Relative Antioxidant Capacity units (ABEL-RAC™). Contact KSL for details on +44(0)1752 565676 or e-mail info@knightscientific.com

REAGENT PREPARATION

All reagents must be equilibrated at room temperature before 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 ascorbic acid are supplied as freeze-dried powders 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 ACCORDING TO THE FOLLOWING INSTRUCTIONS.**

Reconstitution of freeze-dried Pholasin®

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

Load a syringe with 5 mL of Reconstitution-and-Assay Buffer.

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

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.

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

Reconstituted antioxidant Pholasin® is best stored at 2-8°C when not in use (DO NOT FREEZE).

Dispose of needles and syringes using good laboratory practice.

Reconstitution of freeze-dried ascorbic acid

The 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}.

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 ascorbic acid 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.

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

Keep reconstituted bottle in the dark when not in use as the product is light sensitive. Discard any unused ascorbic acid.

Dispose of needles and syringes using good laboratory practice.

STANDARD PREPARATION

In order to create a standard curve to calculate ascorbic acid equivalent units for a sample, a range of ascorbic acid concentrations must be run in the same assay as the sample. This is achieved by pipetting the reconstituted ascorbic acid (1 mmol L^{-1}) and assay buffer directly into the microplate as outlined in the table below.

Volumes of ascorbic acid and R&A buffer added to the microplate well.

1mmol L^{-1} ascorbic acid (µL)	R&A buffer (µL)	Final concentration of ascorbic acid in well (µmol L^{-1})
0	25	0
2	23	10
4	21	20
8	17	40
12	13	60
16	9	80

ASSAY PROTOCOL

For accuracy it is recommended that all controls, standards (if used) and samples be run in duplicate wells.

When using ascorbic acid standards, pipette ascorbic acid and R-&-A buffer into microplate wells as outlined in the Standard Preparation section above (see suggested plate layout below for a potential organisation of microplate wells).

Pipette samples (10 µL) into sample wells. Add 15 µL of R-&-A buffer to make the volume in each well up to 25 µL.

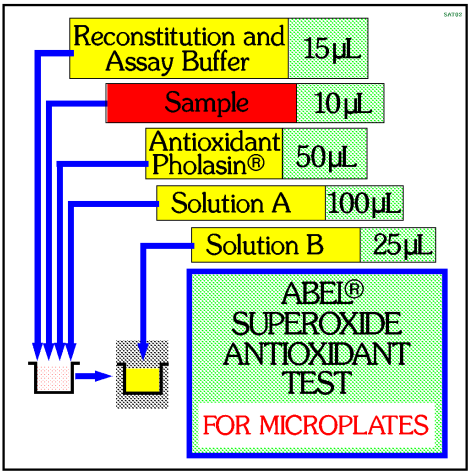
For no-sample controls (or solvent controls) pipette 25 µL of R-&-A buffer into wells.

Pipette Pholasin® (50 µL) and Solution A (100 µL) into all microplate wells.

Incubate the microplate inside the microplate luminometer for 10 minutes before starting reading. Due to the rapid kinetics of the assay each well should be read before the next one is started (known as 'well mode' on some microplate luminometers).

To initiate the generation of superoxide programme the luminometer to automatically inject 25 µL of Solution B into the well 5 seconds after starting the reading. Continue reading for 30 seconds; peak luminescence should be reached after 5 seconds.

Summary of assay protocol (for sample wells) .



Suggested plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	C	C	S1	S1	S2	S2	S3	S3	S4	S4	S5	S5
B	C	C	U1	U1	U2	U2	U3	U3	U4	U4	C	C

C = no-sample control (R-&-A buffer or solvent control); S = ascorbic acid standards (if required); U = sample with unknown antioxidant capacity

CALCULATIONS

Ascorbic acid equivalent units

1. Calculate the maximum (or peak) luminescence recorded for each of the ascorbic acid standards and samples.
2. Plot a standard curve of peak luminescence versus ascorbic acid concentration. See example standard curve below.
3. Determine the µmol L^{-1} ascorbic acid equivalent (AAE) units by using the formula generated to create the standard curve. AAE units are calculated using the line equation from the standard curve using the following designations.

   $y = mx + c$ or $x = (y - c) / m$ where:

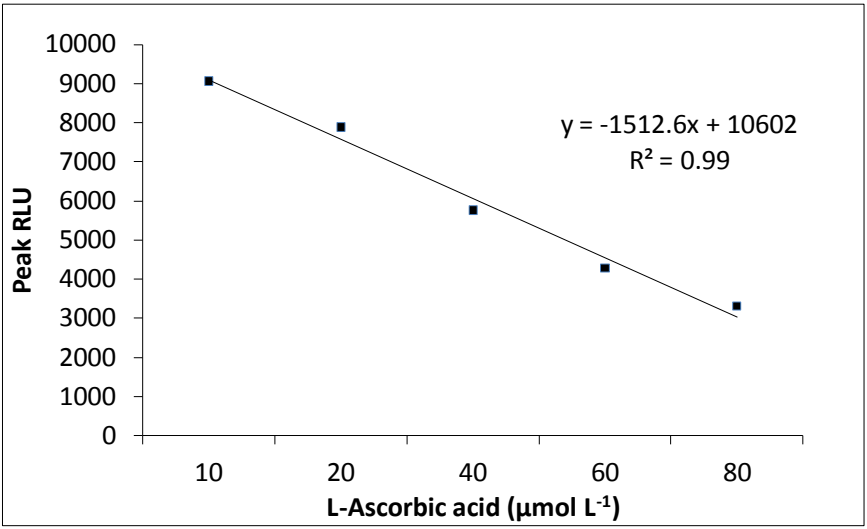
   x = ascorbic acid equivalent units

   y = peak luminescence of sample

   m = slope or gradient of line

   c = y-intercept

4. Sample results can be expressed as AAE units per µL or mg of sample, or per mg of protein (if the protein concentration is known).



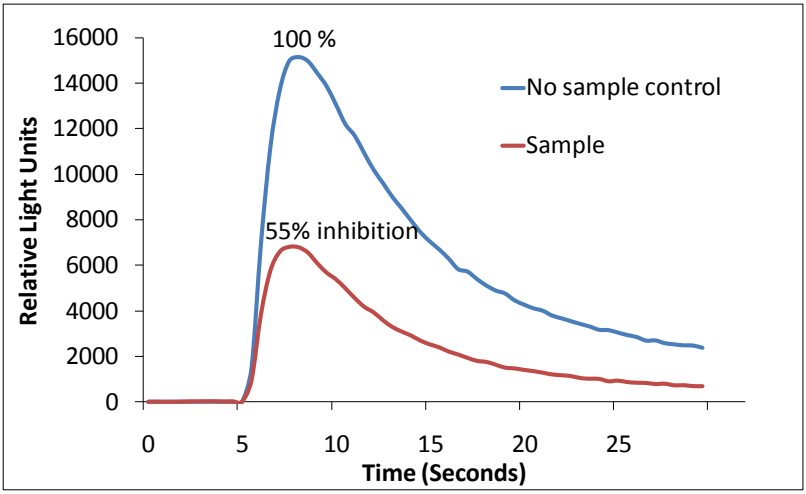
Example standard curve for ascorbic acid standards (final concentrations in well).

% inhibition

1. Calculate the maximum (or peak) luminescence recorded for each sample as well as the no-sample control (or solvent control).
2. Calculate % inhibition of each sample by using the following formula:

   [(peak, control) – (peak, sample)] / (peak, control) x 100

3. The example below shows a sample exhibiting 55% inhibition of luminescence compared to the peak of the no-sample control.



A sample exhibiting 55% inhibition of luminescence compared to control without sample.

ABEL® Relative Antioxidant Capacity (ABEL-RAC™)

Contact Knight Scientific Limited for ABEL-RAC testing protocols and analysis template.

NOTES

The ABEL-60 series is recommended for measuring the antioxidant capacity of samples against enzyme-generated superoxide produced at physiological concentrations. It can also be used to measure superoxide dismutase (SOD) and SOD-mimetic activity.

Contact KSL for details on +44(0)1752 565676 or e-mail info@knightscientific.com

Analysis By ABEL Emitted Light

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