ABEL® ANTIOXIDANT TEST KIT
for HALOGENATED OXIDANTS
with PHOLASIN®

ABEL-31M

A chemiluminescent test for measuring the capacity of a liquid sample to scavenge halogenated oxidants such as hypochlorous acid and the longer-lived derived chloramines. It is also an excellent test for quantifying water and oil soluble ingredients and finished products in cosmetics, foods and nutraceuticals.

CONTENTS

Kit components sufficient for 100 x 200µL tests

1 x bottle Pholasin® (reconstitute to 5mL)

1 x 50mL Reconstitution and Assay Buffer

1 x 25mL Reconstitution Buffer for Chloramine-T

2 x 50mg Chloramine-T (reconstitute to 5mL)

1 x 96 well white luminometer microplate

REAGENT	FORMAT	TEMPERATURE	SHELF LIFE
PHOLASIN (Antioxidant)	Freeze dried	-20°C or lower	up to 12 months
	Reconstituted	DISCARD UNUSED PRODUCT	
RECONSTITUTION & ASSAY BUFFER FOR PHOLASIN	Liquid	-20°C or lower	up to 12 months
	Liquid	2-8°C	up to 1 month
RECONSTITUTION BUFFER FOR CHLORAMINE –T	Liquid	-20°C or lower	up to 12 months
		2-8°C	up to 1 month
CHLORAMINE-T (reconstitute to 5mL)	Powder	2-8°C	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.

Pholasin® is 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.**

INTRODUCTION

In this assay hypochlorous acid, derived from chloramine-T, is used to challenge the test material. This assay is excellent for measuring the antioxidant capacity of materials dissolved/dispersed in both aqueous and organic solvents. This assay is very useful in quantifying antioxidant capacity of materials containing phospholipids, fatty acids, sterols and sphingolipids. Hypochlorous acid attacks the double bond in cholesterol as well as primary amine-containing phospholipids which are oxidized to longer lived oxidants such as the chloramines.

Chloramine-T (4.4mmol L^{-1} final concentration) is added to a cuvette containing: Pholasin®, with and without a 50 µL sample of liquid of unknown antioxidant capacity. Water and oil soluble materials can be tested in this assay. For oils and oil soluble materials acetone and/or alcohol can be used to make the first concentration; further dilutions are carried out with 10% alcohol or acetone. REMEMBER OF USE THE 10% SOLVENT FOR THE NO SAMPLE CONTROL. Other samples include: serum, plasma, synovial fluid, cell supernatants, process water, etc.

If Pholasin® is present when the chloramine-T is injected, light will be emitted; if the sample contains antioxidants capable of scavenging Chloramine-T, then these antioxidants will compete with Pholasin® for the Chloramine-T and less light will be detected. The antioxidant capacity is expressed as the percentage inhibition of light. Controls containing no sample are run with each assay. The inhibition can be further equated to albumin, a well-known scavenger of halogenated oxidants, to obtain these, an albumin standard curve is run as part of the assay (see section Standard curve for albumin).

Samples with pro-oxidant activity are identified when the peak light response of the sample is higher than the no sample control.

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

EQUIPMENT REQUIRED

A microplate luminometer with shaking, temperature control (25°C or ambient) and automatic injectors is ideal. However, if your luminometer does not shake then it is suggested that the microplate should be agitated externally 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. Then carefully remove and dispose of the needle (using good laboratory practice).
5. Replace the screw cap and invert and roll the bottle at least 5 times to dissolve the contents.
6. 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.

Solutions of Chloramine-T

Dissolve Chloramine-T with 5mL of the special buffer. **NOTE:** Chloramine-T is not under vacuum. Discard unused Chloramine-T.

TEST PROCEDURE

Pipette into a well of a white luminometer microplate:

125 µL assay buffer or 75 µL assay buffer + 50µL serum or plasma sample

25µL Pholasin®

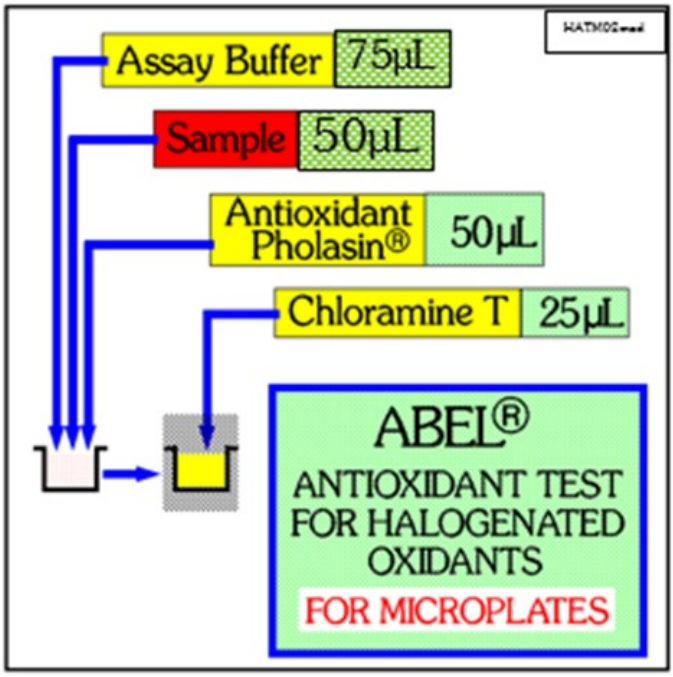
While the well is in the light measuring position, inject 25 µL Chloramine-T to obtain a final concentration of 4.4mmol L^{-1}. Peak luminescence for the control is reached in about 5 seconds whereas the presence of a sample with antioxidant capacity the peak does not usually occur until about 15 seconds. The antioxidant capacity of the sample is expressed as percent inhibition:

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

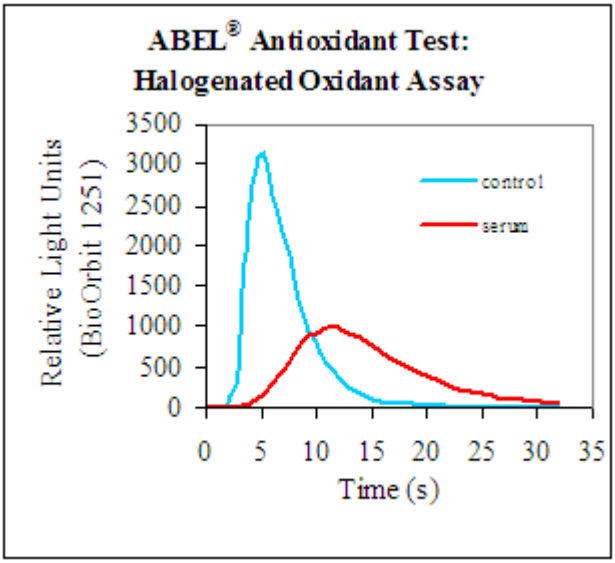
A control without the sample but with an equivalent volume of buffer is run with each assay. The inhibition can be further equated to equivalent albumin concentration, a well-known scavenger of halogenated oxidants.

The kit contains sufficient reagents for 100 tests of 200µL each.

SUMMARY



TYPICAL ANALYSIS

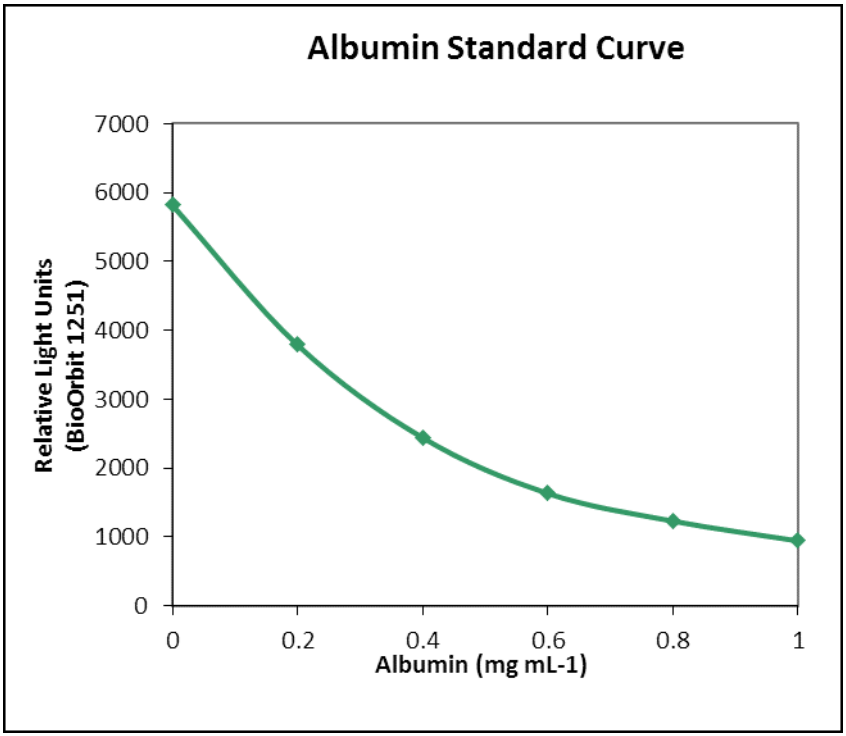


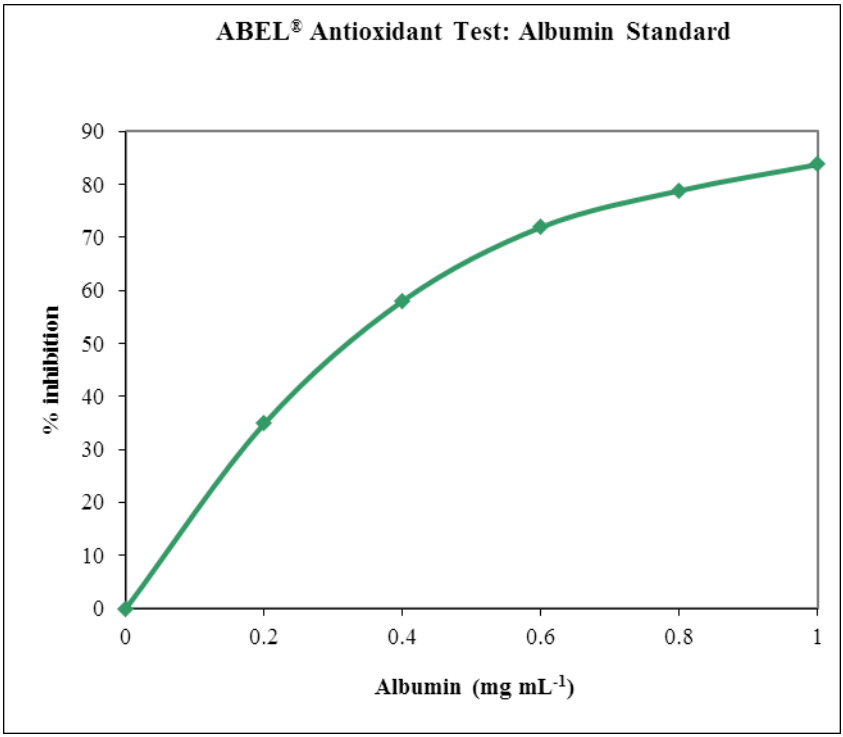
STANDARD CURVE FOR ALBUMIN

1. Make up a 1% solution of albumin (albumin, bovine, SIGMA) in PBS (Phosphate Buffered Saline, pH 7.4). Always use the same source of albumin and always make up fresh solution as it can become a pro-oxidant when it is stored in solution.
2. Pipette into a well: 50 µL Pholasin
   105 µL R&A Buffer
   20 µL of 1% albumin (in PBS) + PBS, as shown in the table below.
3. Inject 25µL Chloramine-T when the microplate well is in the light measuring position.
4. The total volume in the microplate well is 200µL.

Table. Volumes of albumin and PBS added to a microplate well as the sample.

1% Albumin (µL)	PBS (µL)	Final concentration albumin (mg mL^{-1})
0	20	0.0
4	16	0.2
8	12	0.4
12	8	0.6
16	4	0.8
20	0	1.0





Results can be expressed as percent inhibition or emitted light compared to a control, or as albumin equivalent antioxidant units.

Albumin Equivalent Antioxidant Units:

68% inhibition in 50µL sample in a 1:200 dilution ≡ 0.52mg mL^{-1}
→104 mg mL^{-1} albumin equivalent units in pure sample

Analysis By ABEL Emitted Light

For further help and advice, please telephone 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

KNIGHT SCIENTIFIC