TESTING ANTIOXIDANT CAPACITY OF INGREDIENTS AND PRODUCTS
BACKGROUND

ABEL® is an acronym for Analysis By Emitted Light. In the ABEL® antioxidant assays light is generated by a reaction between the photo-protein Pholasin® protein and a variety of reactive oxygen species (ROS). If a material to be tested for potential antioxidant capacity is challenged with one or more of these reactive species in the presence of Pholasin, then any antioxidants in the sample will compete with Pholasin® for these ROS. The result of this competition is a reduction in the amount of light emitted, sometimes referred to as quenching. A light response curve is produced for each sample tested.

The ABEL® (Analysis By Emitted Light) antioxidant test kits, of Knight Scientific Limited, measure the capacity of a sample to scavenge free radicals and other reactive oxygen species (ROS). If the sample has already been exposed to ROS then its antioxidant capacity will have been reduced. Natural materials, such as plants, will have variable antioxidant capacities depending upon conditions under which they were grown as well as the condition of their storage after harvest. Shelf life can also be determined by measuring the loss of antioxidant capacity over time.

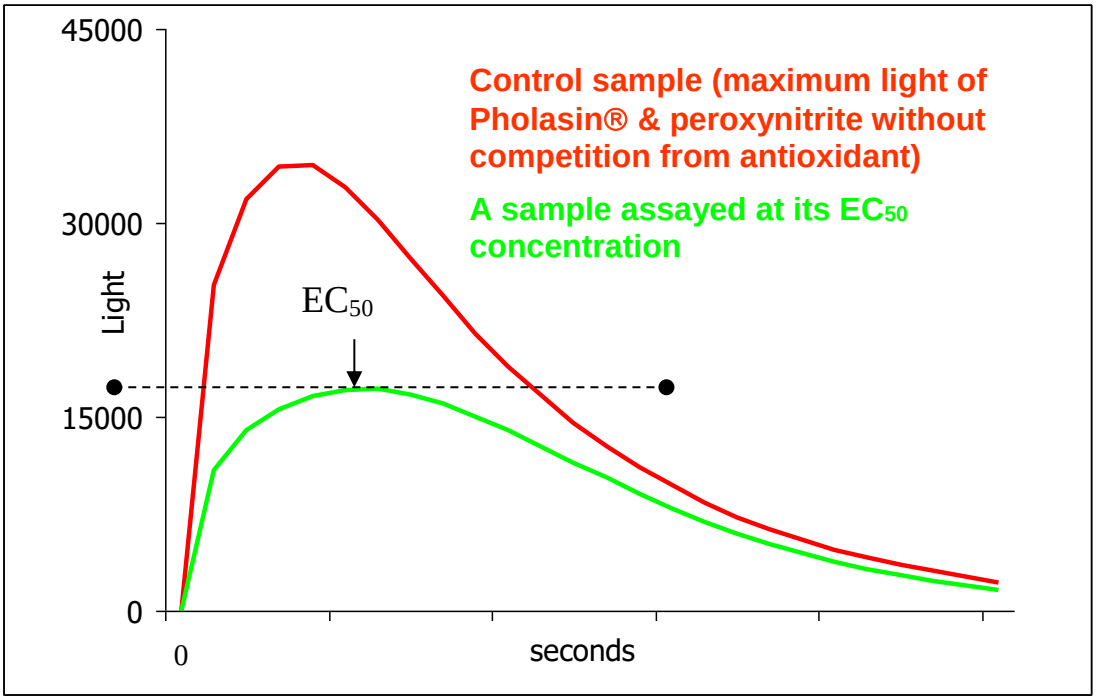
Knight Scientific has developed a highly accurate testing method for quantifying the antioxidant capacity of materials per mg. An ABEL-RAC (relative antioxidant capacity) mg score can be used to adjust the final amounts of ingredients in repeat formulations as well as reject unsuitable ingredients. In this way products based on natural products can be matched batch to batch.

ANTIOXIDANT CAPACITY SCORES: EC_{50} VALUES AND ABEL-RAC mg SCORES

Separate light response curves are produced for each concentration of a material tested as well as the no sample control. The results are presented as EC_{50} values and ABEL-RAC scores. The EC_{50} (effective concentration mg) is the concentration (normalised to g/L or mg/mL) of a material that reduces the light (produced with Pholasin® and the free radical or other reactive oxygen species) by 50%. This reduction in light is the antioxidant capacity of the test material.

The greater the amount of material that is required to reduce the light by half, the weaker the antioxidant capacity. The amount of material required to reduce the light be half is termed the EC_{50} (50% effective concentration). Materials with very high antioxidant capacity have very low EC_{50} values. To make it more readily understandable these EC_{50} values have been converted to positive relative antioxidant capacity scores (ABEL-RAC mg scores) for each free radical or oxidant used to challenge the test material. For examples when peroxynitrite is used as the ROS challenge then the result will be expressed as ABEL-RAC mg Peroxynitrite, for superoxide challenge ABEL-RAC mg superoxide. ABEL-RAC mg scores are the reciprocal of the EC_{50} values multiplied by 100 ($1/EC_{50}$ x 100). The higher ABEL-RAC mg score the higher the antioxidant capacity of the sample.

In the figure below a sample to be tested for antioxidant capacity is challenged with a particular free radical or oxidant in the presence of the light-emitting protein Pholasin®. The concentration that reduced the light by 50% is determined from the results of a range of concentrations and analysed using an exponential regression curve. A template is used to obtain this value. This is the effective concentration (EC_{50}) of the sample. The EC_{50} is converted to an **ABEL-RAC™** score using the formula: **$1/EC_{50}$ (mg) x 100**. ABEL-RAC™ scores, for all the different challenges can be expressed per mg, per dose or percentage in a formula as well as per unit cost.



ABEL-RAC scores are expressed per mg of dried material or µL of a liquid for each of the ROS used to challenge the material. Some materials will be better antioxidants against some free radicals than others.

There are currently six type-specific antioxidant assays, each using a different kind of free radical or non-radical ROS challenge. These are:

- high concentration superoxide assay (ABEL 20 series),
- halogenated oxidant (= hypochlorous acid) assay (ABEL-30 series)
- peroxynitrite assay (ABEL-40 series)
- hydroxyl radical assay (ABEL 50 series)
- enzyme generated superoxide assay (ABEL 60 series)
- peroxyl radical assay (ABEL-70 series)
- singlet oxygen assay (ABEL-80 series)

TYPE- SPECIFIC ANTIOXIDANT ASSAYS

- **ABEL®-20 series: Antioxidant Test using superoxide** and other free radicals. In this assay a bolus of superoxide is produced in the assay, and is used to challenge the test material. This is an excellent assay for measuring the activity of antioxidants such as ascorbic acid that are chemical quenchers of superoxide. The ABEL®-60 series (see below) is recommended for measuring superoxide dismutase (SOD) activity.
- **ABEL®-30 series: Antioxidant Test using halogenated oxidants** such as hypochlorous acid. Hypochlorous acid is produced by activated white blood cells during inflammation. 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.
- **ABEL®-40 series: Antioxidant Test using peroxynitrite**. In this assay, the peroxynitrite anion ($ONOO^-$) is produced continually in the assay[1].where it is used to challenge the test material. Peroxynitrite is a very reactive oxidant that will attack lipophilic antioxidants such as Vitamin E as well as the hydrophilic antioxidant glutathione. Peroxynitrite attacks virtually any antioxidant or molecule with a double bond irrespective of whether the antioxidant is free in solution or bound to other molecules; it will also penetrate cell membranes. In addition to its use in testing ingredients and

[1] Superoxide and nitric oxide are released simultaneously from SIN-1 (3-morpholinosydnonimine hydrochloride) where they react together to produce the peroxynitrite anion ($ONOO^-$).

products it is also used as a total antioxidant capacity (TAC) test for quantifying antioxidant capacity of the blood of people and animals[i].

- **ABEL®-50 series: Antioxidant Test using hydroxyl radicals** produced in the assay to challenge the test material. The hydroxyl radical is the most reactive free radical known and will attack virtually anything in its path. It should, however, be noted that many organic solvents used to dissolve ingredients used in cosmetic preparations, such as ethanol, methanol and DMSO, are very strong quenchers of hydroxyl radical. While it is possible to control for the antioxidant properties of such solvents, the halogenated oxidant and peroxynitrite assays are recommended as the most appropriate assays for materials dissolved in such solvents.
- **ABEL®-60 series: Antioxidant Test for superoxide, superoxide dismutase and mimetics of SOD**. In this assay superoxide is produced continually at precisely controlled rates and reflects the production of superoxide by activated white blood cells as sites of inflammation as well as from xanthine oxidase. It is sensitive enough to measure the amount of superoxide produced by a small number of living cells as well as for quantifying very low concentrations of SOD. Using this assay the antioxidant capacity of an ingredient or product can be expressed as moles of superoxide that can be quenched or as SOD equivalent units. The assay can distinguish between ingredients or products that are quenchers of superoxide and those that are SOD mimetics.
- **ABEL®-70 series: Antioxidant test using peroxyl radicals**. It measures the specific antioxidant capacity of a sample to quench peroxyl radicals. The results are expressed either in moles of peroxyl radical quenched per minute per mg of test material or more usually as ABEL-RAC units per mg.
- **ABEL®-80 series: Antioxidant test using singlet oxygen.** It measures the capacity of a sample to quench singlet oxygen which is generated in the assay. It is an excellent assay in determining the suitability of materials as antioxidants with the potential to prevent lipid oxidation, resulting from singlet oxygen attack.

  [Molecular oxygen, though a free radical, is chemically inert due to its two unpaired electrons in its outermost orbit having the same quantum number which imposes a spin restriction as electrons spinning in the same direction are unreactive. This restriction can be removed by moving one of the unpaired electrons in a way that alleviates this restriction. This mechanism requires input of energy and generates the singlet states of oxygen: Delta singlet oxygen ($^1\Delta_g$ O_2) and Sigma singlet oxygen ($^1\Sigma_g$ O_2) with Delta singlet oxygen the most common in biological systems and usually decays to the Sigma state.]

The scores per mg for each ingredient can be used directly in formulations to determine the theoretical total ABEL-RAC score for the finished product. The theoretical score is then compared to the actual score of the finished product. By determining the ratio of the actual to the theoretical ABEL-RAC score it is possible to quantify positive or negative synergy.

THE TABLE BELOW GIVES A SIMPLE EXAMPLE TO ILLUSTRATE THIS.

INGREDIENTS	ABEL-RAC-mg	PERCENT IN PRODUCT	ABEL-RAC contribution
A	20000	25	5000
B	4000	50	2000
C	7000	20	1400

	SUM		8400
ABEL-RAC	complete	product	14600
	synergy		+1.74

SOME ABEL-RAC peroxynitrite scores determined for particular samples tested

Berberis	6300	Ginger	4000
Bee pollen	9520	Grapeseed extract sample 1	390000
Bilberry	7100	Grapeseed extract sample 2	350000
Broccoli extract	6600	Hawthorn	2600
Cayenne	850	Mint	16000
Curcumin	43000	Picrorhiza	18181
Dandelion	19100	Red sage root	20400
Gallic Acid	122160	Rosemary (second extract)	21500
Gingko leaves	4900	Rosemary (first extract)	819100
Gingko extract	100632	Urtica	30075

These scores are specific to the material actually tested and cannot be used generically for a type of material. Each new batch needs to have its own specific ABEL-RAC mg score and a date of testing.

Dr Jan Knight
Knight Scientific Limited
15 Wolseley Close Business Park
PLYMOUTH, PL2 3BY
Tel: +44 (0)1752 565 676
Fax: +44(0)1752 561 672

Email: jan@knightscientific.com
March 2017