



## Testing the Antioxidant Capacity of Vegan Products and their Ingredients with the ABEL-RAC-Vegan™ Antioxidant Test

### *Certified Vegan Standards*

The Vegan Awareness Foundation requires that products approved to carry the Certified Vegan Logo must:

- not contain meat, fish, fowl, animal by-products (including silk or dyes from insects), eggs or egg products, milk or milk products, honey or honey bee products;
- involve no animal testing of ingredients or finished product by supplier, producer, manufacturer or independent party;
- provide supplier verification that animal products were not used in the manufacturing of ingredients;
- contain no known animal-derived GMOs or genes used to manufacture ingredients or finished products.

Some standard methods used in testing the quality, reproducibility, stability and safety of ingredients and products involve enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase which are either derived from animals or are produced by genetic engineering. Other routine tests, called ELISA (Enzyme-Linked Immunosorbent Assays) used in many laboratories during the research stage of product and ingredient development as well as during the testing of the product for anti-inflammatory properties, involve both enzymes and antibodies. These antibodies are routinely grown in mammals such as rabbits, goats and horses but chickens can also be used for some applications. Methods used for some toxicity tests involve collecting the hemolymph of horseshoe crabs and keyhole limpets. And the routine ATP test for measuring contamination of products or ingredients with bacteria and other pathogens uses the bioluminescent enzyme luciferase from the firefly or a genetically modified version.

The Vegan Awareness Foundation requires that acceptable steps have been taken to thoroughly clean and sanitize surfaces, vessels, utensils and machinery used between vegan and non-vegan production cycles to minimize cross-contamination if shared machinery is used. Would the use of ATP hygiene monitoring systems be considered to an indirect breach of the Certified Vegan Standards?

The Vegan Society is not clear as to whether the use of ATP tests, ELISA tests or toxicity tests using invertebrate hemolymph would contravene vegan standards. However, Knight Scientific Limited uses none of these tools in any way in relation to the preparation or use of its ABEL-RAC-Vegan™ Antioxidant Test

## *Knight Scientific's new ABEL-RAC™ Vegan test for quantifying the antioxidant capacity of raw ingredients and finished products*

Knight Scientific, in appreciating the passion behind the vegan movement, has developed a new antioxidant test, ABEL-RAC™ Vegan, that uses only chemically synthesized reagents and single use plastic laboratory consumables and other laboratory equipment cleaned to prevent cross contamination. The test is based on Knight Scientific's expertise in the field of chemiluminescence, linking emission of light to measurement of free radicals and other non-radical reactive oxygen species (ROS) and the interaction between these ROS and the many phytochemicals in plants that have antioxidant capacity.

ABEL® is an acronym for Analysis By Emitted Light and the resulting ABEL-RAC-Vegan™ score is the antioxidant activity of a mg of material challenged by a cascade of ROS generated sequentially in the test. The light-emitting probe used in the test is luminol, 5-amino-2,3-dihydro-1,4-phthalazinedione. The ABEL-RAC™ (relative antioxidant capacity) Vegan score can be used directly in formulations and in the finished products enabling the identification and final quantification of any possible synergy between individual ingredients.

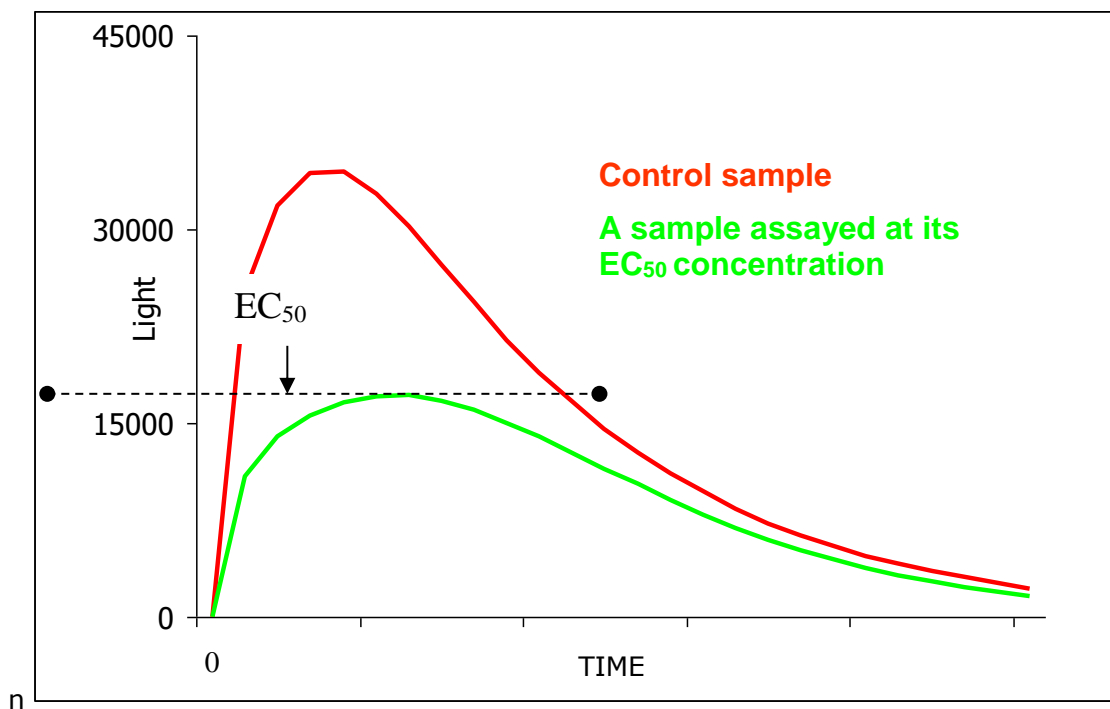
### *Antioxidant Capacity Scores: EC<sub>50</sub> Values and ABEL-RAC-Vegan™ mg scores*

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<sub>50</sub> values and ABEL-RAC Vegan scores. The EC<sub>50</sub> (effective concentration mg) is the concentration (normalized to g/L or mg/mL) of a material that reduces the light by 50%. This reduction in light is then related to the antioxidant capacity of the material being tested. The greater the amount of material required to reduce the light by half, the weaker the antioxidant capacity. Therefore, high EC<sub>50</sub> values indicate low antioxidant capacity. To make it more readily understandable these EC<sub>50</sub> values have been converted to positive relative antioxidant capacity scores (ABEL-RAC™ vegan mg scores) for the total effect of the ROS produced sequentially. ABEL-RAC-Vegan™ mg scores are the reciprocal of the EC<sub>50</sub> values multiplied by 100. The higher ABEL-RAC-Vegan™ mg the higher the antioxidant capacity of the sample. The ABEL-RAC- Vegan™ mg dose is obtained by multiplying the ABEL-RAC- Vegan™ score by the dose in milliliters.

In the figure below a typical sample to be tested for antioxidant capacity is challenged with ROS in the presence of the light-emitting probe luminol. The make up of the different proprietary reagents of Knight Scientific used in the test determine the rate of production and make-up of the ROS. Additional information on the nature of the material being tested can be obtained from examining the kinetics of the reaction between the antioxidant substance to be tested and the ROS.

The graph below has been produced to illustrate the principle of the method. The concentration of test material that reduces the light by 50% is determined from the results of a range of concentrations of material. And while it is theoretically possible to actually use a concentration of sample that reduces the

light by half, most times the results obtained are either above or below the EC<sub>50</sub>. Also, samples with very strong antioxidant capacity can produce almost 100% quenching of light at widely differing concentrations. This effect is easy to identify, which is why the test is first run with a wide range of concentrations followed by a narrow range.



The results are analyzed using an exponential regression curve with a narrow range of concentrations as the assay requires near perfect linearity. The EC<sub>50</sub> is the concentration of the material that reduces the light by 50% compared to the solvent control. The EC<sub>50</sub> value (mg/mL) of the sample is calculated from the equation of a linear regression analysis obtained from plotting sample concentrations (mg/mL) against peak light values obtained from the experimental light response curves. The no-sample control is the amount of light produced in the assay, the 100% value from which a 50% theoretical light quenching value can be calculated. This is the effective concentration (EC<sub>50</sub>) of the sample, the amount of material capable of reducing the light by half. The tests are performed with material diluted many times with the resulting scores taking into account the dilution factor. The stronger the antioxidant capacity of the test sample the smaller the amount of material (the more dilute the sample) is required to produce the EC<sub>50</sub>. Small concentrations such as 10<sup>-10</sup> mg/mL are difficult to comprehend which is how we came to produce the ABEL-RAC™ measure of antioxidant capacity, in which high ABEL-RAC™ scores equate to material with high antioxidant capacity.

### *ABEL-RAC™-Vegan score*

The EC<sub>50</sub>, is converted to an ABEL-RAC™-Vegan score from results obtained in the ABEL-Vegan™ antioxidant capacity test with luminol. The score is calculated from the formula:  $1/EC_{50} \text{ (mg)} \times 100$ . ABEL-RAC-Vegan™ scores, can be expressed per mass (mg or g), per dose, percentage in a formula as well as per unit cost.

ABEL-RAC-Vegan™ scores are expressed per mg of dried material or  $\mu\text{L}$  of a liquid.

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

The following is an example of two ABEL-RAC-Vegan™ scores obtained from two different batches of Echinacea extract. The scores for each batch were obtained initially from five different wide-ranging concentrations of the powered extract in order to determine the order of magnitude of the antioxidant capacity of the material. A narrow range of 4 concentrations of extract were assayed in duplicate, obtaining the peak light response for each concentration.

Using a template to carry out the calculations for Batch A1, the ABEL-RAC-Vegan™ score was 337661 ( $\Xi$  337700) mg units cv 3.2% and when the whole test was repeated on a different day the ABEL-RAC-Vegan™ score for Batch A2 was 328285 ( $\Xi$  328300) mg units cv 0.87%. The mean of Batch A1 and A2 was 332973 (presented as 333000 mg with cv 2%).

A different batch of Echinacea extract, Batch B, was obtained from the same manufacturer a number of months later. The ABEL-RAC-Vegan™ scores for Batch B were determined identically to Batch A with the following results:

ABEL-RAC-Vegan™ score for Batch B1 was 461366 ( $\Xi$  461400) mg units cv 2.2% and for Batch B2, performed 8 days later date 444260 ( $\Xi$  444300) mg units cv 0.1%. The mean of Batch B1 and B2 was 452813 ( $\Xi$ 4538) mg units cv 2.7%.

Batch B was 26.5% stronger than Batch A. This difference could be taken into account in matching new batches of finished product.

*Use of ABEL-RAC™ mg scores in determining the Synergy of Finished Product*

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 hypothetical example to illustrate this.

INGREDIENTS	ABEL-RAC-Vegan™ mg	PERCENT IN PRODUCT	ABEL-RAC-Vegan™ contribution
A	20000	25	5000
B	4000	50	2000
C	7000	20	1400
Predicted score	SUM		8400
ABEL-RAC-Vegan™ Actual score	complete	product	14600
synergy			+1.74

From this information it is possible to further investigate the synergy between the different ingredients in order to maximize the synergy and be able to reproduce new batches in the future.

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