Report:
Cell-based Antioxidant Protection (CAP-e) peroxyl

Client: Trévo LLC

Report number: 77-56-06.2

Date received: July 14, 2011

Date tested: July 26, 2011

CAP-e antioxidant capacity:

Sample	NIS code	Lot/ Batch#	Type of product	Expiration date	CAP-e units (µM GA/mL test product)
Trevo	Trevo	F8	Liquid	N/A	13

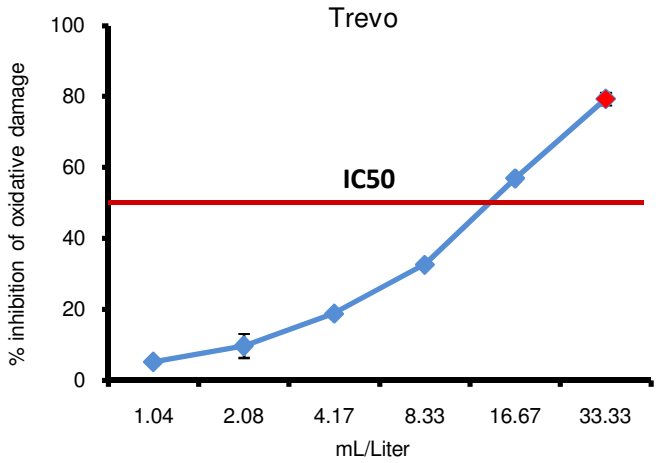
The CAP-e value is in Gallic Acid Equivalent (GAE) units. This measurement reflects the relative antioxidant protection of cells by the test product per volume, compared to the known antioxidant, Gallic Acid.
Protocol reference: NIS/CAPe/AAPH/20090803.

The CAP-e assay is used to test whether natural products contain antioxidants capable of protecting live cells from oxidative damage. Thus, when any protective effect is seen in the CAP-e assay, it shows a biologically meaningful antioxidant protection by the product. In addition, the CAP-e assay is useful for comparing different production lots of the same product and for dose comparison between different test products or ingredients.

The graph below shows the average of each duplicate set of data points for the serial dilutions of the product. For each data point, vertical bars show the standard deviation for each duplicate data set. When duplicate values are almost identical, the standard deviation bars may not be visible.

The IC50 is a measure of the effectiveness of a compound in inhibiting (in the case of the CAP-e assay) oxidative damage. If the product is potent enough to show more than 50% inhibition within the dose range tested, then an IC50 can be calculated.

The point on the graph where the red IC50 line intersects the curve reflects the IC50 dose of the test product, i.e. the dose that provided 50% inhibition of oxidative damage. This IC50 dose is compared to the IC50 dose of the known antioxidant Gallic Acid (which is used as a control in the assay), resulting in a CAP-e value reported in Gallic Acid equivalent units.



Please note: Red data points indicate cell lysing. Cell lysing can happen at higher doses of test products that for various reasons are not well tolerated by the live cells. Lysing can be caused by unfavorable pH, salt concentration and other factors.

PROTOCOL:

For each test product, a 0.5 mL sample of the test product is added to 4.5mL of physiological saline.. Each test product is mixed by inversion and then vortexed. Solids are removed by centrifugation at 2400rpm for 10 minutes. The supernatant of the products is removed and then filtered for use in the CAP-e assay. Serial dilutions are prepared from each filtered supernatant in 0.9% saline at physiological pH.

Red blood cells are treated in duplicate with serial dilutions of a test product. Samples of untreated red blood cells (negative controls) and samples of red blood cells treated with oxidizing agent but not with an antioxidant-containing test product (positive controls) are prepared in hexaplicate. The antioxidants not able to enter the cells are removed by centrifugation and aspiration of supernatant above the cell pellet.

The cells are exposed to oxidative damage by addition of the peroxyl free-radical generator AAPH. Using the indicator dye DCF-DA, which becomes fluorescent as a result of oxidative damage, the degree of antioxidant damage is recorded by measuring the fluorescence intensity of each test sample. The inhibition of oxidative damage is calculated as the reduced fluorescence intensity of product-treated cells, compared to cells treated only with the oxidizing agent. The CAP-e value reflects the IC50 dose of the test products, i.e. the dose that provided 50% inhibition of oxidative damage. This is then compared to the IC50 dose of the known antioxidant Gallic Acid.

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