RESERVE™

The CAP-e Test Results

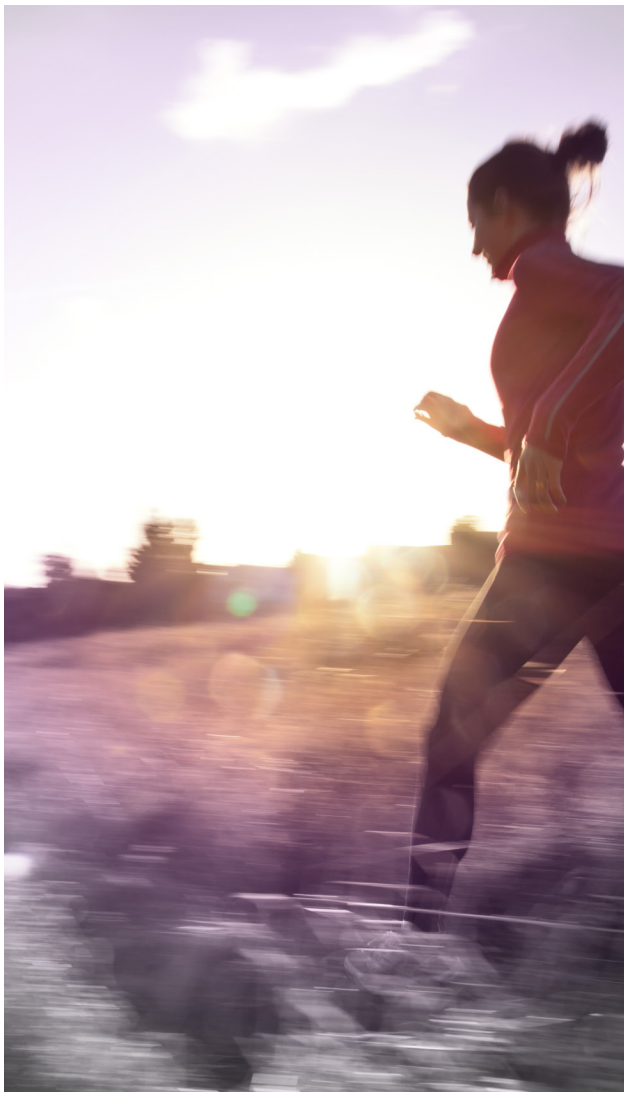
The body of the report is essentially the results section. Please keep in mind, **the purpose of the CAP-e test is to determine if antioxidants are capable of entering into and protecting live cells from oxidative damage**. Since a protective effect was seen and an IC50 was reached, we were able to generate a CAP-e number and state there is extremely significant biological antioxidant penetration and protection by the product.

On a complex product like this, with antioxidant compounds with very different solubility properties, it was agreed that testing in both aqueous and non-aqueous solvent would provide the best appreciation of the antioxidant protection provided to living cells. By doing both extraction methods, you get a broader understanding of the biological activity and antioxidant protection than by doing only one solvent.

We understand that one of the many antioxidant ingredients is resveratrol. This compound has some, but limited solubility in water. It therefore contributes only a little bit to the water extract CAP-e. In contrast, it is likely contributing much more to the ethanol CAP-e.

Other ingredients have other solubility properties.

A separate, additional benefit of the CAP-e is that positive results can point towards further bioassays. Because of the positive test results in the CAP-e of the Jeunesse® RESERVE™ product, NIS Labs will now propose further testing that may generate additional meaningful data.



NIS LABS REPORT:
CELL-BASED ANTIOXIDANT PROTECTION (CAP-e) PEROXYL

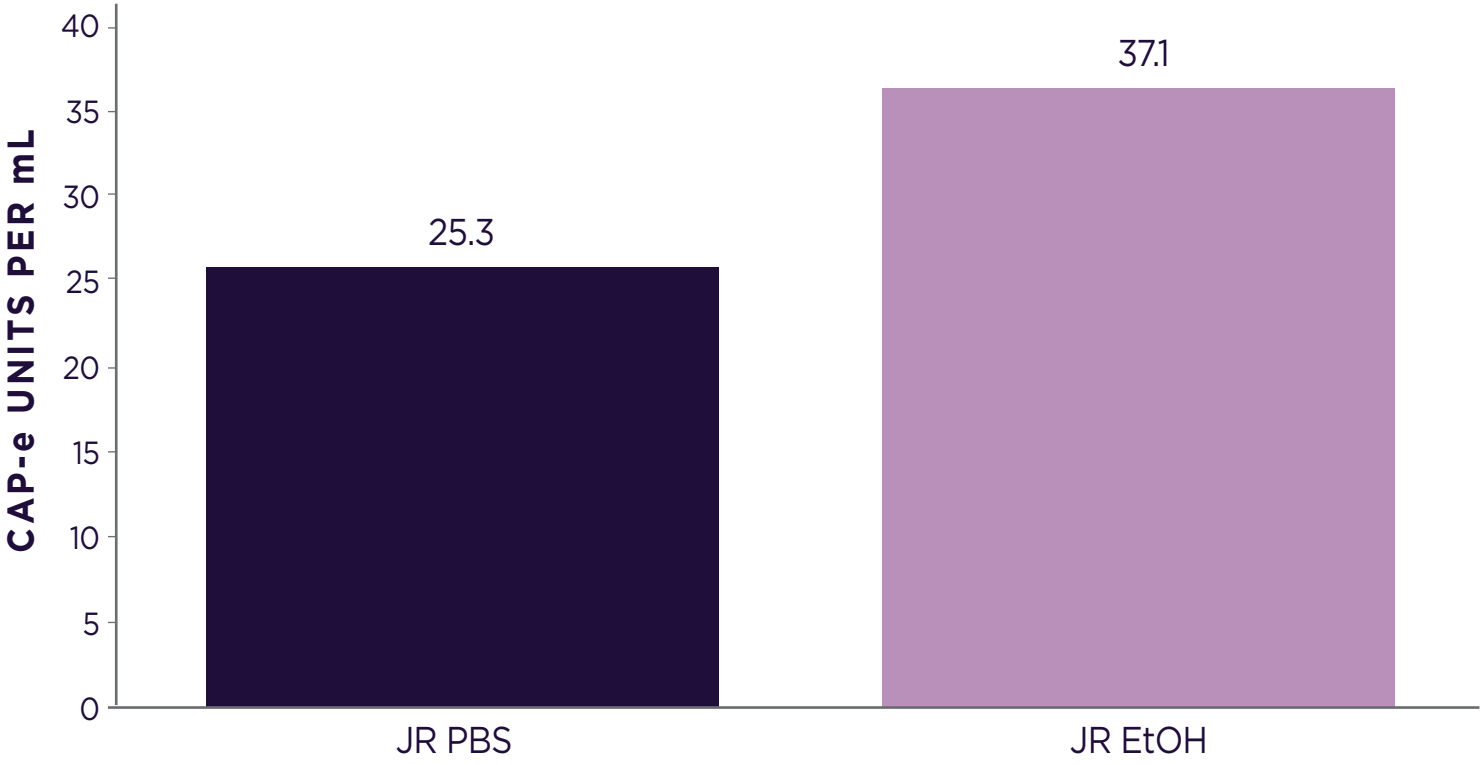
Client: JEUNESSE® Global
Date Received: March 5, 2010
Report Number: 77-0056-03
Date Tested: March 24, 2010

CAP-e ANTIOXIDANT CAPACITY

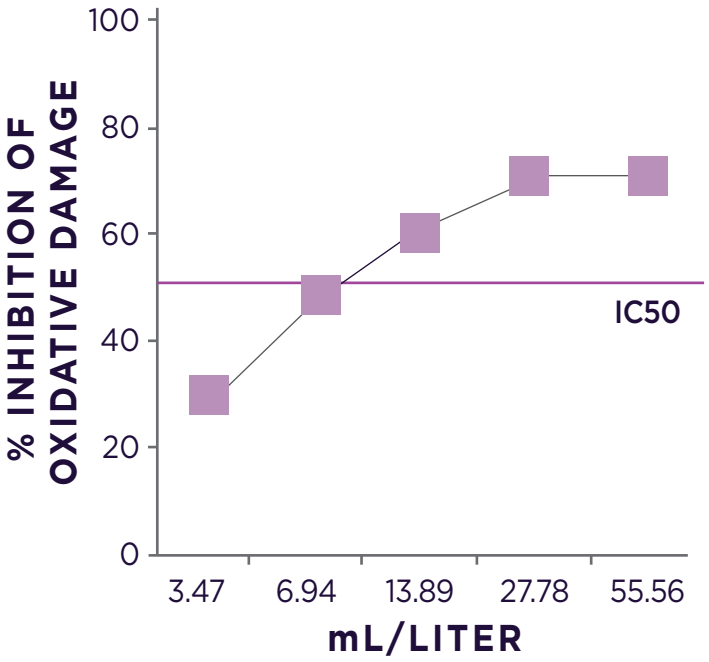
SAMPLE	NIS CODE	LOT BATCH#	TYPE OF PRODUCT	EXPIRATION DATE	CAP-e UNITS (µM GA/mL TEST PRODUCT)
JEUNESSE® RESERVE™	JR PBS	E1	LIQUID	N/A	25.3
JEUNESSE® RESERVE™	JR EtOH	E1	LIQUID	N/A	37.1

The CAP-e value is provided as uM Gallic Acid (GA) per mL liquid test product. This measurement reflects the relative antioxidant protection of cells by the test product per weight, compared to the known antioxidant, Gallic Acid.
Protocol reference: NIS/CAPe/AAPH/20090803.

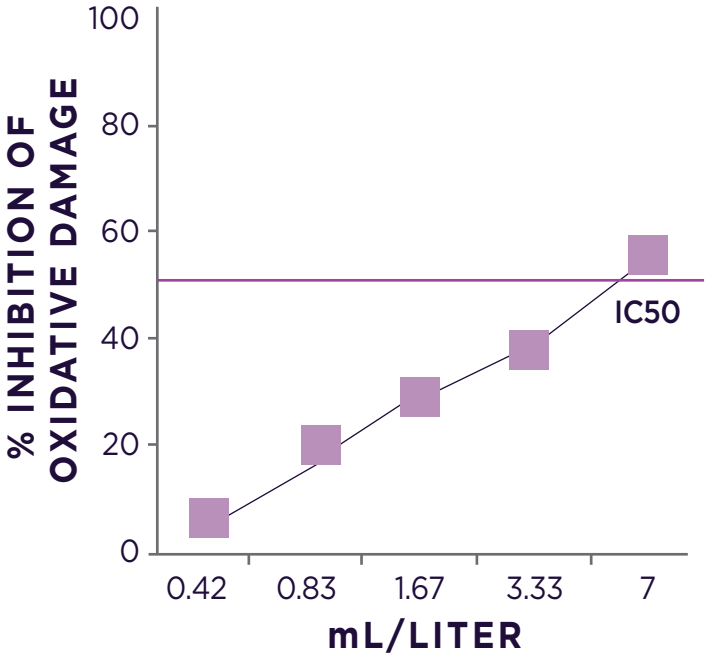
The CAP-e assay is used to test whether natural products contain antioxidants capable of entering into and protecting live cells from oxidative damage. Thus, when any protective effect is seen in the CAP-e assay, it shows a biologically meaningful antioxidant protection by the product. In addition, the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



JR PBS



JR eToh



The complex product Jeunesse® RESERVE™ contains both aqueous and non-aqueous antioxidant compounds. In this testing, extracts were prepared in aqueous solution and ethanol in parallel, to evaluate the antioxidant protection provided to live cells from either extraction method. This data serves to provide more detailed information about the product, and also helps planning of further testing in cell-based models.

The graphs to the left show 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.

PROTOCOL:

For each solvent, a 5 mL sample of the test product is used. Each test product is added to the solvent and 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 were 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.

REVIEWED BY:

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JEUNESSE®

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