

THIRD PARTY CELL-BASED ASSAYS

SOUL CONTINUES TO IMPRESS

Cell-based assays are analytical tests which use cells cultivated in a lab, known as cell lines, as the basis for measuring biological reactions. They have been a bedrock of pre-clinical biomedical research for decades, and provide a crucial role in establishing the direction of clinical investigation.

Cell-based assays provide two primary types of information. First, they reveal the degree of absorption of a substance, such as a phytochemical, into the cell. Second, they measure the degree of action of the substance in relation to a specific outcome, such as the reduction of oxidative damage or inflammation.

Cell-based assays are complementary to in vitro tests, such as the ORAC5 panel. While ORAC measures a “cause” – the antioxidant capacity or potential of a substance – cell-based assays measure an “effect” – how those antioxidants behave in a cellular environment.

Rain International has tested Soul with three cell-based tests which measure primary cellular mechanisms – anti-inflammatory, anti-aging, and antioxidant. In specific, these tests are:



CELLULAR ANTI-INFLAMMATORY ASSAY

Cellular Anti-Inflammatory Assay (NFκB) determines the anti-inflammatory potential of a given material in human cells. NFκB (Nuclear Factor kappa B), a protein complex that is involved in cellular responses to stimuli such as stress and free radicals, is used as inflammation biomarker.

In this particular NFκB assay, Tumor necrosis factor alpha (TNF-α), a pleiotropic inflammatory cytokine, is introduced to the human cells to trigger cellular inflammation. If an anti-inflammatory material presents in the cellular environment, the material inhibits NFκB activation and the degree of inhibition can be monitored via NFκB expression. NFκB expression level of the human cells, treated with and without test materials, under the stressed condition are therefore monitored and compared. Maximum percentage of NFκB expression inhibition induced by tested materials is reported. The concentration used that induced the maximum inhibition of NFκB expression is also noted.”.

- *Bioavailability of anti-inflammatory compounds*
- *As much as a 33% inhibition of cellular inflammation*
- *No cellular toxicity at concentrations tested*

CELLULAR ANTI-AGING ASSAY

Cellular Anti-aging Assay (SIRT1) measures the anti-aging ability of a material using SIRT1 production in human cells as a biomarker for anti-aging. SIRT1 is a protein that is believed to play important roles in longevity and reduction of age-related diseases. Previous studies have shown that when mammals age, SIRT1 expression decreases, where induction and activation of SIRT1 has been associated with extended lifespan. These studies have triggered the search for SIRT1 activators that may be used as dietary supplements to promote health and longevity.

Cellular Anti-aging Assay (SIRT1 assay) determines the ability of a test material to stimulate SIRT1 protein expression in human cells, which translates to the material's anti-aging potential. SIRT1 expression level of human cells treated with and without test materials are compared, and maximum percentage of the SIRT1 expression change is reported. The concentration used that induced maximum percentage of the SIRT1 expression change is noted.

- *Bioavailability of anti-aging compounds*
- *62% increase in the stimulation of the anti-aging enzyme, SIRT1*
- *No cellular toxicity at concentrations tested*

Continued on other side.

CELLULAR ANTIOXIDANT ASSAY

In CAA (Cellular Antioxidant Assay), a fluorescent probe is placed inside of representative human cells, whose loss of fluorescence is an indication of the damage extent from oxygen radical. A material to be tested is incubated with the cells to allow its natural absorption into cells. Then, an oxygen radical inducer is introduced into cellular environment, which triggers the release of oxygen radicals. Without antioxidant material present inside of the cells, oxygen radicals permeate through cell membrane and damage the cells and the marker probe. Such process deters when antioxidant material is present inside of the cells. The cellular antioxidant effect of the test material is then measured by assessing the preservation effect of the marker probe in the presence of the test material absorbed inside of cells.

- *Bioavailability of antioxidant compounds*
- *Significant reduction of free radicals compared to average*
- *No cellular toxicity at concentrations tested*

In each assay, SOUL demonstrated significant positive results at safe concentrations. That is, SOUL significantly reduced radical activity, inhibited the formation of NFkB, and stimulated the expression of SIRT1 in human cell lines.

These results represent an important body of pre-clinical evidence which supports the results of other test, such as ORAC5. They also reflect Rain's ongoing commitment to better understanding Soul's healthful attributes.

ORAC 5.0

There are five predominant reactive species found in the body: peroxy radicals, hydroxyl radicals, peroxynitrite, super oxide anion, and singlet oxygen. Total ORACfn provides a measure of the total antioxidant power of a food/nutrition product against the five predominant reactive species.

The ORAC result is expressed as micromole trolox equivalency (Qmole TE) per gram.

- *Measures antioxidant performance against 5 primary radicals*
- *The industry standard for broad-spectrum antioxidant testing*

SOUL RECEIVES BRUNSWICK LABS GOLD CERTIFICATION

Brunswick Labs, a global leader in antioxidant R&D and testing, has established a test for 'Total ORAC' for Food and Nutrition (Total ORACfn). Total ORACfn represents a breakthrough in comprehensive antioxidant testing for food and nutrition products. Total ORACfn delivers valuable, quantitative analysis to evaluate broad-spectrum antioxidant potential of oil and water-soluble ingredients.

- *A Gold Standard for Consumer Assurance*
- *Evidence of responsible testing*
- *Sets a high competitive threshold*

