Verification Procedure for Reactive Molecules in ASEA™

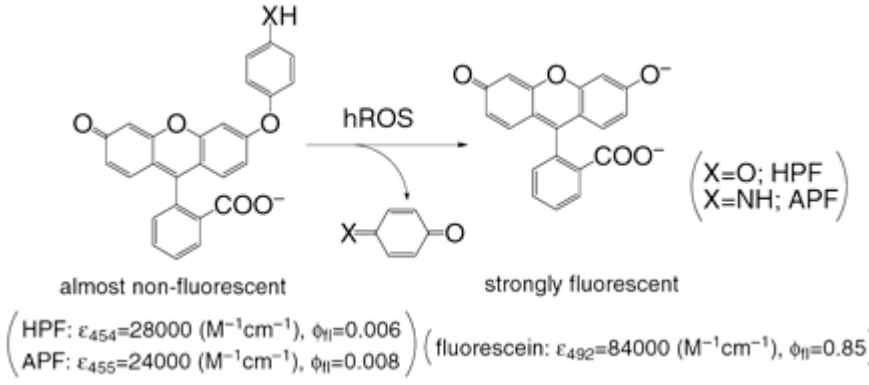
The reactive molecules in ASEA™ are produced by a complex proprietary electrochemical process that reduces and oxidizes the base saline solution, resulting in an equilibrium of several known reactive molecules. These reactive molecules are stable in ASEA™ and measurable using standard analytic methods. Such reactive molecules are the same as those that are naturally produced inside of living cells and have been successfully measured over the last 30 years by the use of certain fluorescent dyes that act as indicators. Verification of the reactive molecules in ASEA™ is done regularly by utilizing three of these same standard fluorescent indicators, namely R-Phycoerythrin (RPE), Aminophenyl fluorescein (APF) and Hydroxyphenyl fluorescein (HPF).

These fluorescent molecules physically change shape (and brightness) when they come into contact with specific reactive molecules. These conformal changes enhance or reduce the fluorescence of the indicators at certain given frequencies of light. This change in fluorescence is then measured using a fluorospectrometer that measures the intensity of the spectrum of light emitted from the indicators.

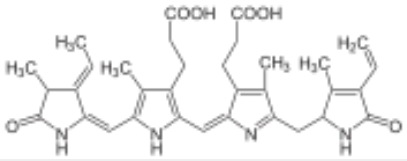
Description of Indicators:

The two new novel probes, Aminophenyl fluorescein (APF) and Hydroxyphenyl fluorescein (HPF) developed by Tetsuo Nagano et. al. (1), are selective for the detection of highly reactive oxygen species (hROS). Both probes have little reactivity towards other forms of ROS.

Assay Principle: Conformal changes in APF and HPF molecules change fluorescent properties.



Phycobiliprotein Fluorescent Dye Spectral Properties.



Fluor	Molecular Weight	Excitation Max (nm)	Emission Max (nm)
R-Phycoerythrin (R-PE)	240,000	480, 545, 565	578

Fig 1: Description of the three indicators used to measure the concentration of reactive molecules in ASEA™. The table on the bottom lists some of the fundamental properties for R-PE.

The change in fluorescence of these indicators before and after exposure to these reactive molecules therefore indicates existence of such reactive molecules. Each of these three indicators is sensitive to specific types of reactive molecules. All of these indicator dyes are very resistant to false positives and are very well studied.

ASEA, LLC uses the services of highly-trained analysts and a Nanodrop™ 3300 fluorospectrometer, by Thermo Fischer Scientific, to routinely measure the amount of reactive molecules found in every batch of ASEA™. In an on-site independent laboratory, almost on a daily basis, one or more of these fluorescent indicators is exposed to ASEA™ and thus the concentration of reactive molecules in ASEA™ is measured. As part of the Quality Assurance process, the concentration of reactive molecules in the "Gold Standard" for ASEA™ is measured along-side each sample. Batches that do not have the concentration of reactive molecules measuring up to the Gold Standard are rejected and discarded. In contrast, the base saline solution always has shown no measurable amounts of reactive molecules before the proprietary electrochemical processing takes place.

Fig. 2 is a screen-shot of a calibration run on the Nanodrop 3300 that shows the response of the R-PE indicator to various concentrations of ASEA™. The differences in reactive molecule concentrations are easily seen on the plots. Up to three measurements are made on each sample concentration to assure consistency. The control base saline solution shows no change in fluorescence, indicating that reactive molecules do not exist in the base saline solution. As the concentration of ASEA™ (diluted by base saline solution) is increased, the change in fluorescence is larger.

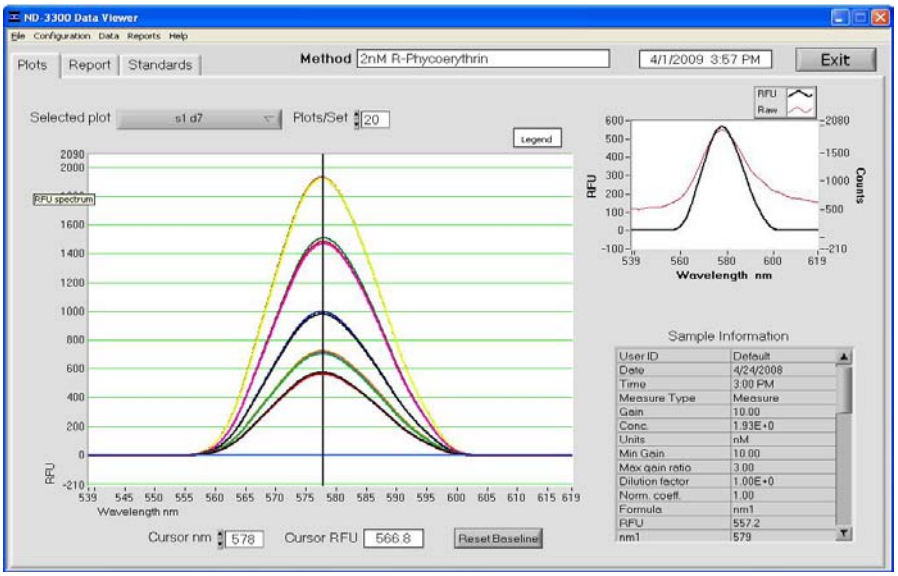


Fig. 2: Screen-shot showing R-PE indicator response to varying concentrations of ASEA™, plots for 15 individual measurements (3 per sample) superimposed. Taken during a calibration run April 2008. Saline solution yields no response and the five different concentrations of ASEA show clearly defined results.

The height of the plots, as shown in Fig. 2, indicate the amount of reactive molecules that exist in the sample. Samples from every batch are measured against the "Gold Standard". Thus every batch of ASEA™ is guaranteed to contain the correct amount and balance of reactive molecules.

In the Quality Assurance laboratory, the highest standard of scientific excellence is maintained. Laboratory methods and procedures were developed by a Ph.D. in Atomic/Medical physics. Equipment is calibrated regularly and indicators are tested against known standards and technicians are trained to recognize potential problems. The existence and concentrations of reactive molecules in ASEA™ has been clearly established. Good scientific data verifies the concentration of reactive molecules that exist in every bottle of ASEA™.