

## Toxicology Endpoint: Reactive Oxygen Species

### Background and Objectives

Reactive oxygen species (ROS) are highly reactive with their surrounding tissue and biological components. The generation of ROS is a good indicator for cell injury induced by a variety of xenobiotic assaults. Excessive ROS-generation can trigger pathological developments such as hypertension and are related to aging processes. Insufficient amounts of ROS can result in reduced host resistance and immune suppression. The determination of a drug's potential to induce ROS is considered a general biomarker of response, provides mechanistic information on xenobiotic stress, and is a crucial component of the early drug development process. Our flow cytometry-based ROS-screen detects the generation of intracellular oxygen radicals and can be combined with changes in membrane integrity and induction of apoptosis.

### Experimental Design

Pharmacelsus measures ROS fluorochromatically in a customizable cell culture system or in a reference cell line (human monocytic leukemia). Several time points can be determined for kinetic analyses, different drug concentrations can be tested for determination of dose-response relationships. A multiplexing with other parameters such as membrane integrity is possible. Pharmacelsus uses a state-of-the-art two-laser, five-color Cytomics FC500 MPL flow cytometer (11 CFR part 11 compliant).



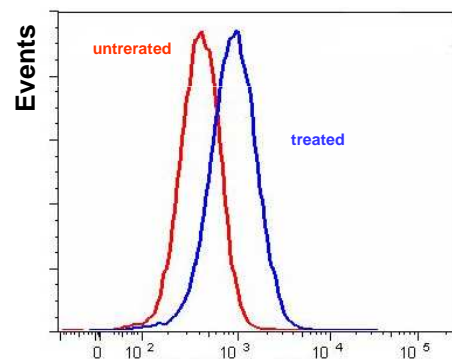
Cytomics FC500 MPL (Beckman Coulter)

### Data acquisition and analysis

At least 10,000 cells will be collected per assay or time point. Cells will be acquired without exclusion of outlayers to collect as much information as possible. If desired, different gating strategies can be applied even post data acquisition. Data will be presented according to international standards as % positive cells and mean fluorescence intensity (MFI). Additional information such as size and granularity can be provided as well.

### Quality benchmarks and controls

Whenever appropriate, positive and/or negative control compounds will be included in each setup. The performance of the flow cytometer will be monitored by measuring predetermined parameters using standardized beads.



**ROS**

Flow cytometric identification of reactive oxygen species (ROS) in cultured human cancer cells

### Supplemental testing

In addition to apoptosis, other parameters such as membrane integrity, apoptosis and cell cycle distribution can be determined by means of flow cytometry.

*Please don't hesitate to contact us for a customized quotation*

**Dr. Reiner Class**  
Head of Target Finding &  
Biological Profiling  
Tel: +49 681 3946-7528  
class@pharmacelsus.de  
www.pharmacelsus.de