



emulate

Protocol for Emulate Organ-Chips:

Total ROS and RNS Assay

July 2, 2019

EP178 v1.0

TITLE Total ROS and RNS Assay	DOCUMENT EP178	VERSION 1.0
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Goals:	Key Steps:	Other Required Materials:
Quantify total reactive oxygen species (ROS) and reactive nitrogen species (RNS) free radical activity from Emulate Organ-Chip effluent samples	<ul style="list-style-type: none"> • Prepare all reagents, samples, and standards • Run the assay • Add stop solution to each well and read the assay plate(s) immediately 	<ul style="list-style-type: none"> • OxiSelect™ In Vitro ROS/RNS Assay Kit (Cell Biolabs #STA-347 or STA-347-5) • Black 96-well plate • Plate reader

Introduction

Accumulation of free radicals, coupled with an increase in oxidative stress, has been implicated in the pathogenesis of several disease states and in the mechanism of action for toxicity of many compounds. This protocol can be used to measure these toxicity endpoints. The OxiSelect™ In Vitro ROS / RNS Assay Kit allows for the measurement of the total amount of free radicals in cell effluent by using a specific ROS / RNS fluorogenic probe. The fluorescence intensity is proportional to the total ROS / RNS levels within the sample.

This protocol uses the Proximal Tubule Kidney-Chip as a reference point. These methods and assay conditions could change with different Organ-Chips.

Method

Sample type	Organ-Chip effluent See protocol EP124 Effluent Sampling.
Recommended assay flow rate	Proximal Tubule Kidney-Chip: 60 µL / h
Recommended effluent dilution	Use undiluted sample
Run assay as described on supplier site	https://www.cellbiolabs.com/in-vitro-ros-rns-assay
Sample concentration detection sensitivity	Minimum 40 nm of hydrogen peroxide within sample. Note: It is recommended to normalize obtained values by protein concentration measured from cell lysates. See protocol EP135 for determining protein concentration from cell lysates.

Validation

The OxiSelect™ In Vitro ROS / RNS Assay Kit has been validated on the Intestine-Chip (Caco-2), Proximal Tubule Kidney-Chip, and the Liver-Chip.

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