CellSimple™ Cellular Reactive Oxygen Species (ROS) Detection Assay Kit

#59496

1 Kit (100 assays)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
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New 02/17

For Research Use Only. Not For Use In Diagnostic Procedures.

Components Ship As: 45670	Item #	Kit Quantity	Storage Temperature
TBHP	13354	50 µL	4°C
Loading Buffer	13438	11 mL	4°C
Phosphate Buffered Saline (PBS-20X)	9808	25 mL	RT

Components Ship As: 13337	Item #	Kit Quantity	Storage Temperature
DCFH-DA	13337	268 µg	-20°C

Description: The CellSimple™ Cellular Reactive Oxygen Species (ROS) Detection Assay Kit is a fluorescent assay designed for use with the CellSimple™ Cell Analyzer. It detects overall ROS levels in living cells. The kit contains the non-fluorescent fluorescein derivative DCFH-DA, a common ROS inducer tert-butyl hydroperoxide (TBHP), Loading Buffer, and 20X PBS wash buffer. DCFH-DA diffuses into cells during pre-incubation and is deacetylated by cellular esterases to form DCFH, which can be rapidly oxidized to the highly fluorescent DCF by cellular ROS. The median fluorescent intensity (MFI) of DCF fluorescence in the green channel (525/45 nm) is used to quantify overall levels of cellular ROS.

Background: Reactive oxygen species (ROS) include a variety of highly reactive oxidant molecules and free radicals that are derived from molecular oxygen. ROS are generated as a result of both normal cellular metabolism and environmental factors including air pollutants or cigarette smoking. The major ROS of physiological significance are superoxide anions ($\bullet O_2^-$), hydroxyl radicals ($\bullet OH$), and hydrogen peroxide (H_2O_2) (1-3). Low to moderate ROS levels play important roles in normal physiological processes including vascular tone regulation, oxygen sensing, immunological reaction, regulation of translation, and signal transduction. High ROS concentrations can lead to adverse modifications of cellular components, such as carbohydrates, lipids, proteins, and DNA. Many of these modifications have been linked to diseases including atherosclerosis, diabetes, cancer, and neurological disorders. As a result, regulation of reducing and oxidizing (redox) state is not only critical for cell health, but is also an attractive therapeutic target for many diseases (1-3).

2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) is a non-fluorescent fluorescein derivative used as a non-ionic, non-polar, cell membrane permeable dye. After crossing the cell membrane, DCFH-DA is hydrolyzed by intracellular esterases to a non-fluorescent dichlorodihydrofluorescein (DCFH). DCFH is relatively more polar and cell membrane-impermeable and thus accumulates in cells. In the presence of cellular ROS, DCFH is oxidized to the highly fluorescent dichlorofluorescein (DCF). The fluorescent intensity of DCF is proportional to the overall cellular ROS level (4,5). These fluorescent signals can be detected using the Open Flow Cytometry application on the CellSimple™ Cell Analyzer.

CellSimple™ Cell Analysis System: The CellSimple™ Cell Analyzer is a benchtop instrument that utilizes a disposable thin-film cassette and a combination of a 488 nm laser, two photomultiplier tubes (525/45 nm and 561 nm LP filters), Coulter Principle-based cell measurements, and on-board software to provide easy-to-run applications and data analysis. Data acquisition occurs within approximately 10 seconds per test. The instrument relies on disposable cassettes for sample handling, which alleviates the need for flow cell cleaning and fluidics maintenance and the instrument is small enough to be portable between the lab bench and the hood. Applications include quantitative assessments of cell viability, apoptosis, other labeled antibody markers and single and multiplexed bead-based assays for protein and cellular analysis.

Specificity/Sensitivity: The CellSimple™ Cellular Reactive Oxygen Species (ROS) Detection Assay Kit is expected to detect reactive oxygen species in living cells across all species. A cell number and DCFH-DA titration is recommended to be performed when developing an assay with your cells of interest.

Storage: All components in this kit are stable for at least 6 months when stored at the recommended temperature and left unused. Upon receipt, #9808 should be removed from kit box and stored at room temperature. #13337 should be stored at -20°C. Remaining components should be stored at 4°C.

Background References:

(1) Alfadda, A.A. and Sallam, R.M. (2012) *J Biomed Biotechnol* 2012, 936486.

(2) Birben, E. et al. (2012) *World Allergy Organ J* 5, 9-19.

(3) Bolisetty, S. and Jaimes, E.A. (2013) *Int J Mol Sci* 14, 6306-44.

(4) Wang, H. and Joseph, J.A. (1999) *Free Radic Biol Med* 27, 612-6.

(5) Eruslanov, E. and Kusmartsev, S. (2010) *Methods Mol Biol* 594, 57-72.

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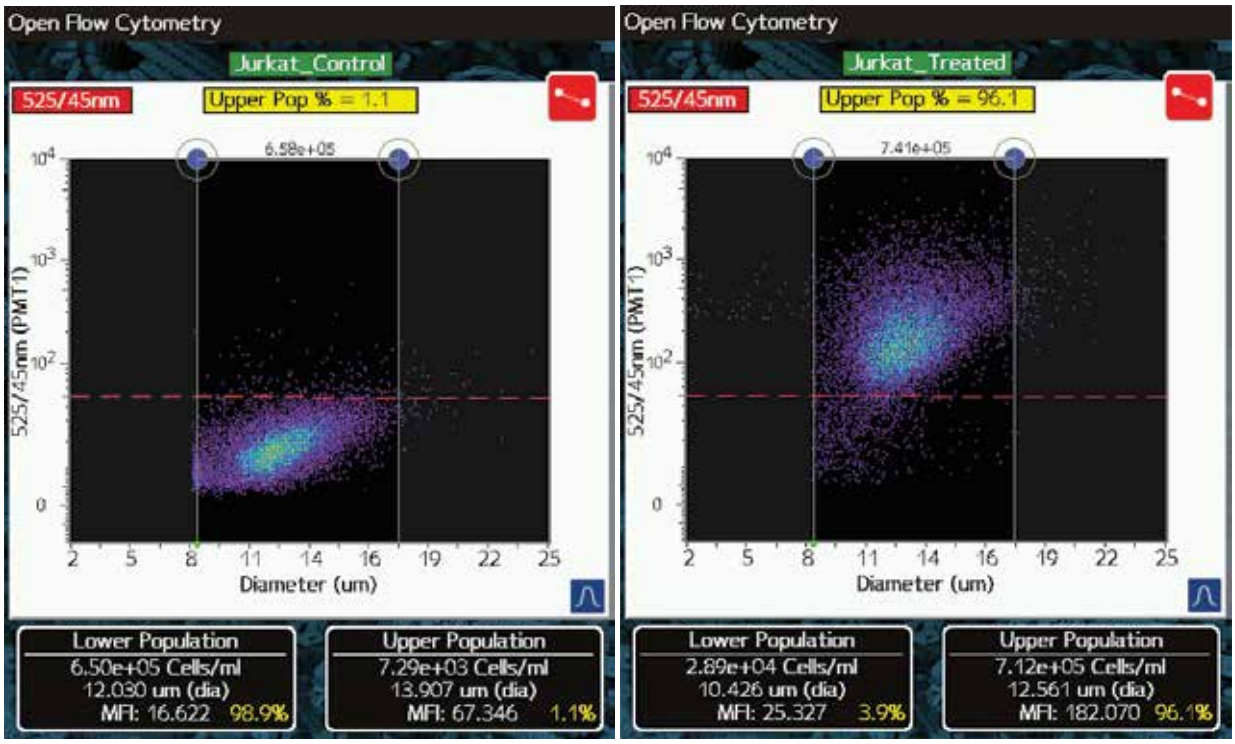
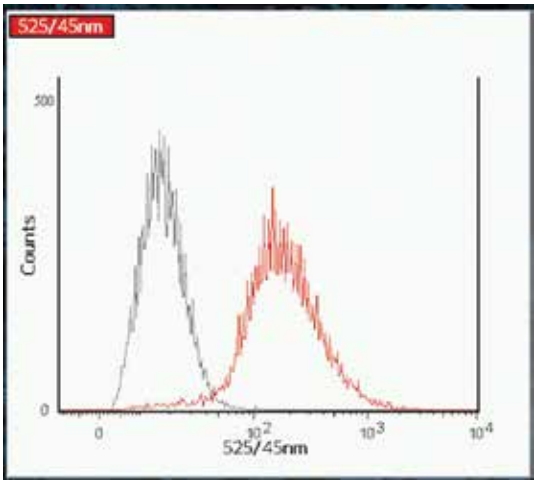
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Untreated **Treated**



CellSimple™ cell-based analysis of live Jurkat cells that were labeled with DCFH-DA (10 µM, 37°C, 30 min) and untreated (left panel) or TBHP-treated (1 mM, 37°C, 1 hour; right panel) using the CellSimple™ Cellular Reactive Oxygen Species Assay Kit. Data was collected in the green (525/45 nm) channel and analyzed on the Open Flow Cytometry application. Instrument screen shots are shown.



Median Fluorescence Intensity (MFI) of DCFH-DA labeled (10 µM, 37°C, 30 min) live Jurkat cells untreated (black) or treated with TBHP (10 mM, 37°C, 1 hour; red) shown above in a histogram overlay. The MFI shift in the green channel (525/45 nm) demonstrates the increase of Reactive Oxygen Species within the cell.

Please note your screen may look slightly different from the screen shots on the data sheet due to variations between software versions.

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CellSimple™ Cellular Reactive Oxygen Species (ROS) Detection Assay

A. Instrumentation: The CellSimple™ Cellular Reactive Oxygen Species (ROS) Detection Assay kit was specifically designed for use with the CellSimple™ Cell Analyzer.

B. Kit components:
- DCFH-DA
- TBHP
- Loading Buffer (10X)
- Phosphate Buffered Saline (PBS-20X)

C. Additional reagents needed, but not supplied:
- DMSO
- Reverse osmosis/deionized (RODI) water or equivalent
- Cell culture media

D. Reagent preparation

Note: Allow all reagents to reach room temperature before use.

1. 1X PBS: To prepare 500 mL 1X PBS add 25 mL PBS-20X to 475 mL RO/DI water, mix.
2. 1X Loading Buffer: To prepare 110 mL 1X Loading Buffer add 11 mL Loading Buffer (10X) to 99 mL RODI water, mix.
3. 10 mM DCFH-DA Stock Solution: Add 55 µL DMSO to the vial of DCFH-DA to make a 10 mM stock solution.
4. 10 mM TBHP Working Solution: Add 1.0 µL TBHP Stock Solution (vortex stock solution before adding) to 700 µL cell culture media, mix.

Notes:
- DCFH-DA lyophilized powder is stable for up to 12 months at -20°C if protected from light and moisture. 10 mM DCFH-DA Stock Solution should be used within 6 months after reconstitution and should be aliquoted and stored at -20°C protected from light. Close cap tightly after each use. Any aqueous dilution of the DCFH-DA stock should be used within the same day.
- For optimal labeling in different cells lines, we recommend first performing an assay titrating the DCFH-DA at a final concentration between 5 to 20 µM, for both suspension and adherent cell lines.

E. Protocol for suspension cells

1. Suspend cells in pre-warmed media at 1 x 10^6 cell/mL. Prepare 0.5 mL aliquots of the cell suspension into centrifuge tubes, where each tube is one assay sample point. (Example: for three experimental samples plus one positive and one negative (unlabeled) control, a total of 5 x 0.5 mL = 2.5 mL will be needed.)
2. Dilute 10 mM DCFH-DA Stock Solution 1:1000 in pre-warmed (37°C) 1X Loading Buffer for a final concentration of 10 µM DCFH-DA. We recommend preparing 0.5 mL of 10 µM DCFH-DA per sample. (Example: for 5 total samples, add 2.5 µL of the DCFH-DA stock solution to 2.5 mL of pre-warmed 1X Loading Buffer.)
3. Centrifuge cell suspension at 1200 rpm for 5 min. Aspirate the media and pipet 0.5 mL of the DCFH-DA Labeling Solution (made in step 2) to each tube. Re-suspend cells by gently pipetting up and down. Incubate at 37°C for 30 minutes.
4. Centrifuge cells at 1200 rpm for 5 min, then remove the labeling solution.
5. Wash cells once with 1.0 mL pre-warmed 1X PBS (warmed to 37°C), repeat step 4.
6. Re-suspend cells, by gently pipetting up and down, in 0.5 mL pre- warmed cell culture media (warmed to 37°C) per tube. Perform experimental perturbations and incubate cells for desired time.
7. For the positive control, re-suspend cells in 1mM TBHP (dilute 10 mM TBHP Working Solution 1:10 in warm cell culture medium; for example, 50 µL Working Solution added to 450 µL media). Incubate at 37°C for 60 minutes.
8. Centrifuge cells at 1200 rpm for 5 minutes then remove the supernatant.
9. Wash cells once with 1.0 mL pre-warmed 1X PBS, repeat step 8.
10. Re-suspend cells by trituration into 0.5 mL pre-warmed 1X PBS.
11. Analyze 75 µL on the CellSimple Cell Analyzer using the Open Flow Cytometry Application, selecting only the 525/45 nm detection channel. Please see the CellSimple user guide for more details about using the Open Flow Cytometry Application.

F. Protocol for adherent cells

1. Plate cells in a 12 well TC plate (1.0 mL cells per well) in cell culture media (warmed to 37°C), place in incubator overnight to allow cells to adhere to the surface. A typical cell number is between 1.0 x 10^5 and 5.0 x 10^5 cells/mL. A cell number titration may be necessary for optimal results.
2. Dilute 10 mM DCFH-DA Stock Solution 1:1000 in pre-warmed (37°C) 1X Loading Buffer for a final concentration of 10 µM DCFH-DA. We recommend preparing 0.5 mL of 10 µM DCFH-DA per sample. (Example: for 4 samples plus one positive and one negative (unlabeled) control, the total volume is 3.0 mL. Add 3.0 µL of the 10 mM DCFH-DA stock solution to 3.0 mL of pre-warmed 1X Loading Buffer.)
3. Aspirate the cell culture media from the assay plate and wash wells once with 0.5 mL pre-warmed 1X PBS. Aspirate the 1X PBS and add the DCFH-DA Labeling Solution (prepared in step 2) and incubate at 37°C for 30 minutes.
4. Aspirate media from the plate, wash cells once with 0.5 mL pre- warmed 1X PBS then aspirate the 1X PBS.
5. Perform experimental perturbations and incubate cells at 37°C for desired time.
6. For the positive control, replace PBS with 1 mM TBHP (dilute 10 mM TBHP Working Solution 1:10; for example: add 50 µL of 10 mM TBHP solution to 450 µL media.) Incubate at 37°C for 1 hour.
7. Aspirate media from the plate, wash cells once with 0.5 mL pre- warmed 1X PBS then aspirate the 1X PBS.
8. Add 0.5 mL of pre-warmed cell dissociation media to each well to detach cells, incubate for 5-10 minutes at 37°C.
9. Place cell suspension into separate centrifuge tubes and centrifuge cells at 1200 rpm for 5 minutes.
10. Remove the supernatant, then wash cells once with 0.5 mL pre- warmed 1X PBS repeat step 7.
11. Re-suspend cells into 0.5 mL pre-warmed 1X PBS.
12. Analyze 75 µL with the CellSimple™ Cell Analyzer using the Open Flow Cytometry Application selecting only the 525/45 nm detection channel. Please see the CellSimple™ user guide for more details about using the Open Flow Cytometry Application.