

Assay kits

ROS Assay Kit


Reactive Oxygen Species Detection Assay Kit (ROS)

Protocol


IMPORTANT NOTES – Before you begin

- ✓ Each ROS Assay Kit is suitable for approximately 300 assays in 96-well plates or 150 assays in 24-well plates
- ✓ Prefer using black 96-well/clear bottom plates for fluorescence measurement
- ✓ We recommend to always including a positive control sample (cells treated with TBHP for 2-4 hours) to your experiment
- ✓ Allow reagents to reach room temperature before beginning
- ✓ Avoid direct exposure to light
- ✓ We do not recommend preparing DCF-DA solution for less than 10 assays in 96-well plate (corresponding to 5 assays in 24-well plates).
- ✓ After addition of DCF-DA, ROS activity can be visualized under fluorescence microscopy or measured by cytometry analyses.

For additional information and protocols (optimization, scaling, co-transfection...) tips, troubleshooting or other applications

 www.ozbiosciences.com

Any questions?

 tech@ozbiosciences.com

ROS Detection Assay Kit | Specifications

Package content	ROS0300: 25mM DFC-DA in DMF (35µL), 10X ROS Buffer (5 mL), Tert-Butyl Hydrogen Peroxide (TBHP) 25 mM (50 µL) Number of assays (96-well plate): 300 Number of assays (24-well plate): 150
Shipping conditions	The kit is shipped at +4°C.
Storage conditions	Upon reception, store the DFC-DA tube and the 10X ROS Buffer at -20°C. Avoid multiple freeze/thawing cycles. Store all other components at 4°C
Shelf life	1 years from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures. During long term storage or upon shipping in cold weather.

Protocol

The following protocol is given for titration of ROS production in adherent and suspension cells in 96-well plate.

1. Solution preparation

1X ROS Buffer. Dilute the 10X ROS Buffer 1:10 by adding 9 volumes of distilled H₂O to 1 volume of 10 X ROS Buffer. Store the solution at **4°C**.

NOTE: This solution can be kept at **4°C** for several months.

DCF-DA Solution. Prepare DCF-DA solution extemporaneously by diluting the DFC-DA (25mM) solution into 1X ROS Buffer according to the Table 1 below.

Number of assay in 96-well plate (100 µl)	Number of assays in 24-well plate (200 µL)	DCF-DA 25mM (µL)	1X ROS Buffer (mL)	Total DCF-DA solution (mL)
10	5	1	1	1
50	25	5	5	5
100	50	10	10	10

Table 1: Volumes to consider for preparing DCF-DA Solution

Tert-Butyl Hyrogen Peroxide (TBHP) solution (positive control). Fresh working solution should be made for every assay and discard after use. Prepare a 250 µM TBHP working solution by diluting 25 mM TBHP stock solution into your cell culture medium. The final concentration of TBHP to use depends on the cell type and sensitivity: depending on the cell, we recommend to use concentrations between 5 and 50 µM TBHP.

2. Fluorescent measurement of ROS production

Protocol for Adherent cells

Cell preparation

NOTE: We recommend using a black plate with clear bottom for an enhanced fluorescence measurement.

- Seed cells in a 96-well plate under standard culture conditions.
- Carry out experiment by adding chemical compounds or biological agents to cells.
- Keep untreated wells for a 2 or 4 hours stimulation with TBHP (positive control).
- 2 to 4 hours prior the experiment, stimulate cells with the desired TBHP concentration.

Measuring ROS

- Remove culture medium and gently wash cells using **100 µL** PBS.
- Repeat this step one time.
- Stain the cells by adding **100 µL** of diluted DCF-DA solution.
- Incubate cells with DCF-DA solution for **30 min** at **37°C** in the dark.
- Remove DCF-DA solution and gently wash cells using **100 µL** PBS.
- Add **100 µL/well** PBS and read fluorescence immediately.
- Measure fluorescence using a fluorescence microtiter plate reader (**exc: ~485nm/ em: ~535nm**).
- Subtract background fluorescence of the non-treated cells from all other values.

Protocol for Suspension cells

1. Cell preparation

NOTE: Cells should be healthy and density should not exceed 1×10^6 cells/mL

- a. Cultivate your cells under standard culture conditions.
- b. Carry out experiment by adding chemical compounds or biological agents to cells and prepare supplemental wells for TBHP stimulation.
- c. 2 to 4 hours prior the experiment, stimulate cells with the desired TBHP concentration.

2. Measuring ROS

- a. Harvest sufficient volume of cells to reach $\sim 1 \times 10^5$ cells per sample into 1.5 mL tubes.
- b. Wash cells by centrifugation (**5min x 500g**) using PBS.
- c. Repeat this step one time and remove supernatant.
- d. Resuspend the cell pellets in **200 μ L** of DCF-DA solution.
- e. Incubate cells with DCF-DA solution for **30 min** at **37°C** in the dark.
- f. Centrifuge cells (**5min x 500g**) and remove supernatant containing DCF-DA solution.
- g. Resuspend cells in **100 μ L/tube** PBS and place them into black microtiter plate.
- h. Measure fluorescence using a fluorescence microtiter plate reader (**exc: 485nm/em: 535nm**).
- i. Subtract background fluorescence of the non-treated cells from all other values.

IMPORTANT NOTE

At step g., a 20 μ L aliquot can be applied onto a microscope slide for fluorescent microscopy visualization. Protect samples from light.

NOTES

NOTES

Additional products

- **OZBlue Cell Viability kit** for cell viability measurement
- **MTT Cell Proliferation Assay Kit** for measuring toxicity and cell viability

Purchaser Notification

Limited License

The purchase price paid for ROS Detection Assay Kit by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). These reagents are intended **for internal research only** by the buyer. Such use is limited to the use in the product manual. Furthermore, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

Purchasers may terminate this License at any time by returning all ROS Detection Assay Kit material and documentation to OZ Biosciences, or by destroying all ROS Detection Assay Kit components. Purchasers are advised to contact OZ Biosciences with the notification that a ROS Detection Assay Kit is being returned in order to obtain a refund and/or to expressly terminate a research only license granted through the purchase of the kit(s).

This document covers in full the terms of the ROS Detection Assay Kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The ROS Detection Assay Kit and all its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

EUROPE & ASIA OZ Biosciences SAS

163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France

Ph: +33 (0) 486 948 516
Fax: +33 (0) 463 740 015

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com



USA & CANADA OZ Biosciences INC

7975 Dunbrook Road
Suite B
San Diego CA 92126
USA

Ph: + 1-858-246-7840
Fax: + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com