BIOLUMINOR

DCFH-DA for Detection of ROS

Table 1 Contents and storage

Material	Amount	Storage	Stability
DCFH-DA	50mg 100mg	♦ -20℃ ♦ Desiccate ♦ Protect from light	When stored as directed, products are stable for at least 1 year
Approximate fluorescence excitation/emission maxima: 480/530 nm.			

The assay employs the cell-permeable fluorogenic probe 2', 7'-Dichlorodihydrofluorescin diacetate (DCFH-DA). In brief, DCFH-DA is diffused into cells and is deacetylated by cellular esterases to non-fluorescent 2', 7'-Dichlorodihydrofluorescin (DCFH), which is rapidly oxidized to highly fluorescent 2', 7'-Dichlorodihydrofluorescein (DCF) by ROS.

Accumulation of reactive oxygen species (ROS) coupled with an increase in oxidative stress has been implicated in the pathogenesis of several disease states. The role of oxidative stress in vascular diseases, diabetes, renal ischemia, atherosclerosis, pulmonary pathological states, inflammatory diseases, and cancer has been well established. Free radicals and other reactive species are constantly generated in vivo and cause oxidative damage to biomolecules, a process held in check by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. Measuring the effect of antioxidant therapies and ROS activity intracellularly is crucial to suppressing or treating oxidative stress inducers.

Cell Biolabs' OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence) is a cell-based assay for measuring hydroxyl, peroxyl, or other reactive oxygen species activity within a cell. The assay employs the cell-permeable fluorogenic probe 2', 7'-Dichlorodihydrofluorescin diacetate (DCFH-DA). In brief, DCFH-DA is diffused into cells and is deacetylated by cellular esterases to non-fluorescent 2', 7'-Dichlorodihydrofluorescin (DCFH), which is rapidly oxidized to highly fluorescent 2', 7'-Dichlorodihydrofluorescein (DCF) by ROS (Figure 1). The fluorescence intensity is proportional to the ROS levels within the cell cytosol. The effect of antioxidant or free radical compounds on DCF-DA can be measured against the fluorescence of the provided DCF standard. The kit has a DCF detection sensitivity limit of 10 pM. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

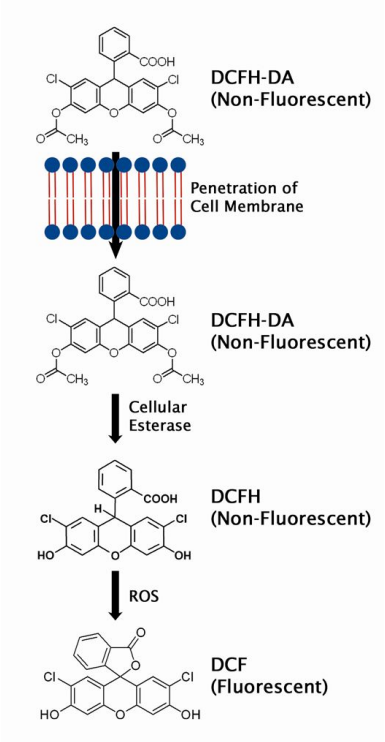

Fig. 1 Mechanism of DCF Assay

Assay Principle

The OxiSelect™ Intracellular ROS Assay Kit is a cell-based assay for measuring antioxidant or ROS activity. Cells are cultured in a 96-well cell culture plate and then pre-incubated with DCFH-DA, which is cell-permeable. The unknown antioxidant or ROS samples are then added to the cells. After a brief incubation, the cells can be read on a standard fluorescence plate reader. The ROS or antioxidant content in unknown samples is determined by comparison with the predetermined DCF standard curve.

Materials Required but Not Provided

1. Sterile DPBS for washes and buffer dilutions
2. Hank's Balanced Salt Solution (HBSS)
3. Cell culture medium (ie: DMEM +/-10% FBS)
4. 96-well black or fluorescence microtiter plate
5. Fluorescent microplate reader capable of reading 480 nm (excitation) and 530 nm (emission)

Storage

Upon receipt, store the DCFH-DA and DCF Standard at -20ºC. Avoid multiple freeze/thaw cycles. Store the Cell Lysis Buffer and Hydrogen Peroxide at 4ºC.

Preparation of Reagents

• 1X DCFH-DA: Dilute the 20X DCFH-DA stock solution to 1X in cell culture media, preferably without FBS. Stir or vortex to homogeneity. Prepare only enough for immediate applications.

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Notes:

• 1X DCFH-DA/media solution contains 5% methanol. For cells that are sensitive to methanol, we recommend instead preparing a 0.1X (100 µM) solution of DCFH-DA in cell culture media.

• Due to light-induced auto-oxidation, DCFH-DA solutions at any concentration must be protected from light.

• Hydrogen Peroxide (H2O2): Prepare H2O2 dilutions in DMEM or DPBS as necessary. Do not store diluted solutions. Hydrogen Peroxide may be used as a positive control in the assay, or as a cell treatment.

Preparation of Standard Curve

1. Prepare a 1:10 dilution series of DCF standards in the concentration range of 0 µM – 10 µM by diluting the 1 mM DCF stock in cell culture media (see Table 1).

Standard Tubes	DCF Standard (µL)	Culture Medium (µL)	DCF (nM)
1	10	990	10,000
2	100 of Tube #1	900	1000
3	100 of Tube #2	900	100
4	100 of Tube #3	900	10
5	100 of Tube #4	900	1
6	100 of Tube #5	900	0.1
7	100 of Tube #6	900	0.01
8	0	1000	0

Table 1. Preparation of DCF Standards

2. Transfer 75 µL of each DCF standard to a 96-well plate suitable for fluorescence measurement. Add 75 µL of the 2X Cell Lysis Buffer.

3. Read the fluorescence with a fluorescence plate reader at 480 nm excitation /530 nm emission.

Assay Protocol

I. DCF Dye Loading

1. Prepare and mix all reagents thoroughly before use. Each unknown sample should be assayed in duplicate or triplicate.

2. Culture cells in either a clear or black 96-well cell culture plate.

Note: If using a black plate, choose an appropriate plate based on your fluorometer's reader (i.e. choose a clear bottom black plate for bottom readers).

3. Remove media from all wells and discard. Wash cells gently with DPBS or HBSS 2-3 times. Remove the last wash and discard.

4. Add 100 µL of 1X DCFH-DA/media solution to cells. Incubate at 37ºC for 30-60 minutes.

5. Remove solution. Repeat step three using multiple washes with DPBS or HBSS. Remove the last wash and discard.

6. Treat DCFH-DA loaded cells with desired oxidant or antioxidant in 100 µL medium.

II. Quantitation of Fluorescence

• Fluorescence microscopy or Flow cytometry: Fluorescence can be analyzed on an inverted fluorescence microscope or by flow cytometry using excitation and emission wavelengths of 480 nm and 530 nm, respectively.

• Fluorescence Plate Reader: • Assays performed in black cell culture fluorometric plates: Plate may be read immediately for kinetic analysis or after 1 hour for static analysis. Plates read for kinetic analysis may be read in increments of 1 and 5 minutes up to 1 hour or more as necessary. Read the fluorescence with a fluorometric plate reader at 480 nm/530 nm.

• Assays performed in clear cell culture plates: After treatment with desired oxidant or antioxidant, carefully remove treatment media from all wells and discard. Wash cells gently with DPBS or HBSS 2-3 times. Remove the last wash and discard. Add 100 µL of medium to each well. Add 100 µL of the 2X Cell Lysis Buffer, mix thoroughly and incubate 5 minutes. Transfer 150 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read the fluorescence with a fluorometric plate reader at 480 nm/530 nm.

References

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