ROS Detection Cell-Based Assay Kit (DCFDA)

Item No. 601520

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. After opening kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
10009322	Cell-Based Assay Buffer Tablet	1 ea	RT
601521	DCFDA Assay Reagent	1 vial/25 µl	-20°C
601522	Pyocyanin Assay Reagent	1 vial/2 µmols	-20°C
601292	N-acetyl Cysteine Assay Reagent	3 vials/10 mg	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

NOTE: It is recommended that gloves be worn at all times when working with mitochondrial inhibitors.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorescent plate reader with a monochromator, or with filter sets capable of measuring an excitation wavelength between 480-500 nm and emission wavelength between 510-550 nm. Alternatively, a flow cytometer equipped with a blue laser (488 nm) and a filter for measuring FITC (530/30 nm).
2. A black, clear-bottom tissue culture-treated 96-well plate.
3. A V-bottom, 96-well dilution plate (suspension cell protocol).
4. A centrifuge with microplate adapter capable of >400 × g.
5. A source of pure, glass distilled, or HPLC-grade water. *NOTE: Ultra-Pure water is available for purchase from Cayman (Item No. 400000).*
6. Non-denatured ethanol.
7. Microcentrifuge tubes.

INTRODUCTION

About This Assay

2,7-Dichlorofluoroscin Diacetate (DCFDA or 2,7-dichlorodihydrofluoroscein diacetate) is a widely-used, cell-permeable redox sensitive fluorescent probe that is oxidized by ROS and certain RNS to yield the highly fluorescent product 2,7-dichlorofluoroscein. Cayman's ROS Detection Cell-Based Assay Kit (DCFDA) uses DCFDA as a fluorescent probe for the detection of ROS generation. Pyocyanin, a cell-permeable compound capable of redox cycling, is included as a positive control for ROS generation. N-acetyl-cysteine is included as an antioxidant control.

PRE-ASSAY PREPARATION

Reagent Preparation

1. **Cell-Based Assay Buffer Tablet - (Item No. 10009322)**

   Dissolve tablet in 100 ml of pure water to make Cell-Based Assay Buffer. Unused Cell-Based Assay Buffer can be stored at 4°C for up to one year.

2. **DCFDA Reagent - (Item No. 601521)**

   This reagent is supplied as a 10 mM solution of DCFDA in DMSO. Thaw and use as detailed below to make ROS Staining Buffer. Once opened, DCFDA Reagent can be stored at -20°C for up to one month. Avoid freeze thaw cycles.

3. **Pyocyanin Assay Reagent - (Item No. 601522)**

   This reagent is supplied as a powder. Dissolve pyocyanin in 200 µl of non-denatured ethanol to yield 10 mM solution. Pyocyanin solution can be stored at -20°C for up to one month.

4. **N-acetyl Cysteine Assay Reagent - (Item No. 601292)**

   This reagent is supplied as a 10 mg powder of N-acetyl cysteine. Store unused vials at -20°C until kit expiration.

Pre-Assay Preparation

1. **ROS Staining Buffer**

   In a separate tube, dilute DCFDA Reagent (10 mM) in Cell-Based Assay Buffer to obtain a working concentration between 10 µM-50 µM, depending on cell line (*For example:* 20 µl DCFDA into 20 ml of Cell-Based Assay Buffer for 10 µM). Any unused ROS Staining Buffer should be discarded immediately after use.

2. **Pyocyanin Working Reagent**

   In a separate tube, dilute 10 µl of the Pyocyanin Assay Reagent (10 mM) into 100 µl of Cell-Based Assay Buffer. This will yield a 1 mM Working Reagent of pyocyanin. Unused Pyocyanin Working Reagent should be discarded immediately after use.

3. **N-acetyl Cysteine Working Reagent**

   In the vial provided, dissolve 10 mg vial of N-acetyl cysteine in 200 µl of Cell-Based Assay Buffer to make a 300 mM N-acetyl Cysteine Working Reagent. Any unused N-acetyl Cysteine Working Reagent should be discarded immediately after use.

ASSAY PROTOCOL

For Adherent Cells

1. Plate cells at desired concentration in a black tissue culture treated 96-well plate and culture cells per desired protocol in the media best suited for your cell line. Ensure that cells are healthy and not overgrown. *(NOTE: When working with unfamiliar cell lines, we recommend performing a seeding titration as cell types can vary in size and volume).*
2. Designate wells as positive (Pyocyanin) and negative controls (N-acetyl cysteine) (we recommend a minimum of two replicates for each condition).
3. Carefully aspirate off the culture media and carefully add ~150 µl of Cell-Based Assay Buffer.
4. Carefully aspirate Cell-Based Assay Buffer leaving a small amount (~10-20 µl) of liquid in the well.
5. Add 130 µl of ROS Staining Buffer to each well.
6. Add 10 µl N-acetyl Cysteine Assay Reagent to designated negative control wells.
7. Cover plate and incubate for 30 minuntes at 37°C - *protected from light*.
8. Following the 30 minute incubation, add 10 µl of the Pyocyanin Working Reagent to designated positive control wells and incubate for an additional hour at 37°C - *protected from light*.
9. Carefully aspirate Staining Buffer and add 100 µl of Cell-Based Assay Buffer.
10. Place Assay Plate on fluorescent plate reader and measure the fluorescence using an excitation wavelength between 480-500 nm and an emission wavelength between 510-550 nm.

For Suspension Cells (V-bottom 96-well plate*)

1. Culture cells per desired protocol in media best suited for your cell line.
2. Add cell suspension at the desired concentration to a V-bottom 96-well plate. Ensure that cells are healthy and not overgrown. *NOTE: When working with unfamiliar cell lines, we recommend performing a seeding titration as cell types can vary in size and volume.*
3. Designate wells as positive (Pyocyanin) and negative controls (N-acetyl Cysteine). We recommend a minimum of two replicates for each condition.
4. Centrifuge the plate at 400 x g to pellet cells.
5. Without disrupting the cell pellet, carefully remove the culture media and wash with ~150 µl of Cell-Based Assay Buffer.
6. Centrifuge the plate at 400 × g to pellet cells.
7. Without disrupting the cell pellet, carefully remove off the Cell-Based Assay Buffer, leaving a small amount (~10-20 µl), and add 130 µl of ROS Staining Buffer to each well.
8. Add 10 µl N-acetyl Cysteine Assay Reagent to designated negative control wells.
9. Cover plate and incubate for 30 minutes at 37°C - *protected from light*.
10. Following the 30 minute incubation, add 10 µl of the Pyocyanin Working Reagent to designated positive control wells and incubate for an additional hour at 37°C - *protected from light*.
11. Centrifuge the plate at 400 × g to pellet cells.
12. Carefully remove ROS Staining Buffer and add 100 µl of Cell-Based Assay Buffer.
13. Readout:
    a. **Plate reader -** Transfer cells to a black, tissue culture-treated 96-well plate. Place Assay Plate on fluorescent plate reader and measure the fluorescence using an excitation wavelength between 480-500 nm and an emission wavelength between 510-550 nm.
    b. **Flow cytometer readouts -** Transfer cells to tubes appropriate for your flow cytometer. DCFDA is typically excited with a 488 nm laser and emits in the FITC channel. Collect at least 20,000 events.

**NOTE: If a V-bottom 96-well plate or centrifuge microplate adapter is unavailable, volumes found in this protocol are applicable to 1.5 ml microfuge tubes.*

ANALYSIS

Performance Characteristics

ROS generation is represented as total DCFDA fluorescence. It is important that positive and negative controls be included in every experiment for all cell types tested. Data can be expressed as total fluorescence, geometric mean fluorescence (flow cytometry), or as % of controls.

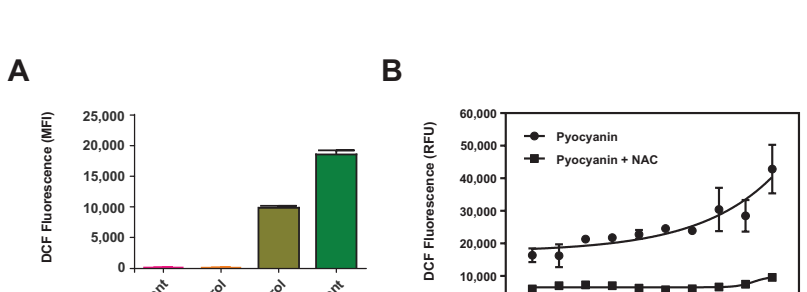

Figure 1. *Panel A:* THP1 cells treated with control compounds and analyzed via flow cytometry *Panel B:* A concetration response curve of MCF7 cells treated with pyanocyanin in the presence and absence of 2 nM N-acetyl Cysteine.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
No fluorescence or minimal fluorescence is detected	A. Cells are not at sufficient density B. Gain is not optimized	A. Conduct seeding titrations to determine optimal cell density before performing experiment B. Adjust gain to optimize signal

Reference

1. LeBel, C.P., Ischiropoulos, H., and Bondy, S.C. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chem. Res. Toxicol.* **5(2)**, 227-231 (1992).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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