Version 1 Last updated 5 July 2018

ab236206 DHE (Dihydroethidium) Assay Kit - Reactive Oxygen Species

For the measurement of ROS in live cells.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

DHE (Dihydroethidium) Assay Kit - Reactive Oxygen Species (ab236206) measures ROS directly in live cells. This kit uses DHE as a fluorescent probe for the detection of ROS generation and is specific for superoxide and hydrogen peroxide. Antimycin A, an inhibitor of complex III of the mitochondrial electron transport chain, is included as a positive control for ROS generation. N-acetyl Cysteine is included as an anti-oxidant control.

ROS generation is represented as total DHE 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 % or controls.

Prepare cells. Designate positive and negative wells.
(centrifuge if using suspension cells)

Aspirate culture media and add 150 µl Cell-based Assay Buffer
(centrifuge if using suspension cells)

Aspirate most of Cell-based Assay Buffer then add ROS Staining Buffer to each well

Add N-Acetyl Cysteine Assay Reagent to negative wells

Cover plate and incubate for 30 minutes at 37°C protected from light

Add Antimycin A Working Reagent to positive wells and incubate for a further hour at 37°C protected from light.
(centrifuge if using suspension cells)

Aspirate ROS Staining Buffer and add Cell-Based Assay Buffer

Measure fluorescence using excitation 480-520 nm and emission 570-600 nm
For Flow measure at 488 nm on PE channel. Collect ≥20,000 events

2. Materials Supplied and Storage

Store kit at -20°C immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Antimycin A Assay Reagent	1 vial	-20°C	-20°C
Cell-Based Assay Buffer Tablet	1 tablet	RT	+4°C
Dihydroethidium Assay Reagent	2 x 20 µL	-20°C	-20°C
N-acetyl Cysteine Assay Reagent	2 x 10 mg	-20°C	-20°C

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex = 480-520 nm, Em = 570/600 nm
- Flow cytometer with blue laser (488 nm) and filter for measuring PE (575/26 nm)
- 96 well plate with clear flat bottom, preferably black
- V-bottom, 96-well dilution plate (suspension cell protocol)
- Centrifuge with microplate adapter capable of >400 x *g.*

Δ Note: If a V-bottom plate or centrifuge microplate adapter is unavailable volumes found in this protocol are applicable to 1.5 ml centrifuge tubes.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Antimycin A Assay Reagent

1. Keep on ice whilst in use.
2. Prepare a 150 µM Antimycin A Working Reagent by diluting 15 µl of Antimycin A Assay Reagent into 1 mL of Cell-Based Assay Buffer in a separate tube.
3. Antimycin A Assay Reagent can be stored at -20°C for up to six months.
4. Discard any Working Reagent immediately after use.

5.2 Cell-Based Assay Buffer Tablet

1. Dissolve table in 100 mL of ultrapure water to make Cell-Based Assay Buffer.
2. Unused Cell-Based Assay Buffer can be stored at +4°C for up to one year.

5.3 Dihydroethidium Assay Reagent

1. Ready to use as supplied.
2. Thaw and use to make ROS staining buffer.
3. Once opened Dihydroethidium Assay Reagent can be stored at -20°C for up to one month.
4. Avoid freeze thaw cycles.

5.4 N-acetyl Cysteine Assay Reagent

1. Dissolve 10 mg vial of N-acetyl Cysteine in 200 µL Cell-Based Assay Buffer to make a 300 mM N-acetyl Cysteine Working Reagent.
2. Discard any unused Working Reagent after use.

5.5 ROS Staining Buffer

1. Dilute Dihydroethidium 1:1000 in Cell-Based Assay Buffer to prepare a working concentration of 5 µM (15 µL Dihydroethidium into 15 mL of Cell-Based Assay Buffer).
2. Discard any unused ROS Staining Buffer after use.

6. Assay Procedure

– It is important to conduct optimization experiments for every cell type and experimental condition as cell types often vary.

6.1 For Adherent Cells (Black plate):

1. Plate cells at desired concentration, per desired protocol, in a media best suited to your cell line. Ensure that cells are healthy and not overgrown.
   Δ 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 (Antimycin A) and negative controls (A-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 minutes at 37°C protected from light.
8. Following the 30-minute incubation, add 10 µL of the Antimycin A Working Reagent to designated positive control wells and incubate for an additional hour at 37°C protected from light.
9. Carefully aspirate ROS Staining Buffer and add 100 µL of Cell-Based Assay Buffer.
10. Place plate on fluorescent plate reader and measure the fluorescence using an excitation wavelength between 480-520 nm and an emission wavelength between 570-600 nm.

6.2 For Suspension Cells (V-bottom plate):

1. Culture cells per desired protocol in media best suited for your cell line.
2. Add cell suspension at desired concentration to a V-bottom plate. Ensure that cells are healthy and not overgrown.
   Δ 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 (Antimycin A) and negative controls (A-acetyl Cysteine) (we recommend a minimum of two replicates for each condition).
4. Centrifuge the plate at 400 x *g* to pellet cells.
5. Carefully aspirate off the culture media without disrupting the pellet and carefully add ~150 µL of Cell-Based Assay Buffer.
6. Centrifuge the plate at 400 x *g* to pellet cells.
7. Carefully aspirate Cell-Based Assay Buffer without disrupting the pellet, leaving a small amount (~10-20 µL) of liquid in the well.
8. Add 130 µL of ROS Staining Buffer to each well.
9. Add 10 µL N-acetyl Cysteine Assay Reagent to designated negative control wells.
10. Cover plate and incubate for 30 minutes at 37°C protected from light.
11. Following the 30-minute incubation, add 10 µL of the Antimycin A Working Reagent to designated positive control wells and incubate for an additional hour at 37°C protected from light.
12. Centrifuge the plate at 400 x *g* to pellet cells.
13. Carefully aspirate ROS Staining Buffer without disrupting pellet and add 100 µl of Cell-Based Assay Buffer.
14. For Plate reader: Transfer cells to a black plate on fluorescent plate reader and measure the fluorescence using an excitation wavelength between 480-520 nm and an emission wavelength between 570-600 nm.
15. For Flow cytometry: Transfer cells to tubes appropriate for your flow cytometer. DHE is typically excited with a 488 nm laser and emits in the PE channel. Collect at least 20,000 events.

Δ Note: If a V-bottom plate or centrifuge microplate adapter is unavailable volumes found in this protocol are applicable to 1.5 ml centrifuge tubes.

7. FAQs / Troubleshooting

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

8. Typical Data

Data provided **for demonstration purposes only.**

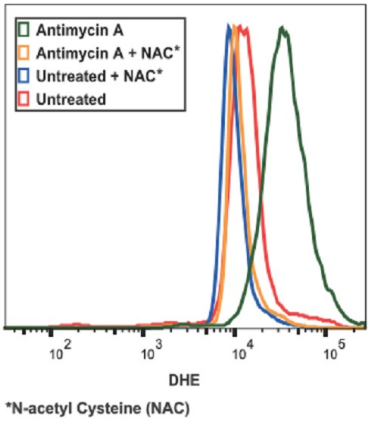

Figure 1. THP-1 cells (human monocytic leukemia cell line) treated with controls and stained using the protocol for suspension cells.

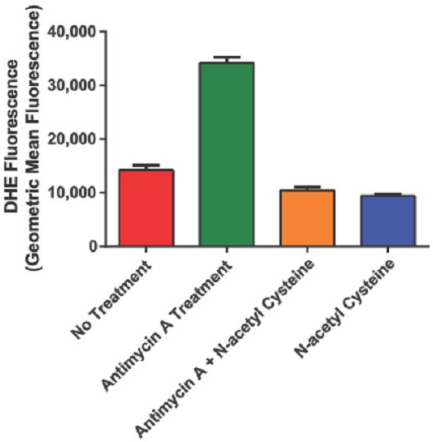

Figure 2. THP-1 cells (human monocytic leukemia cell line) treated with controls and stained using the protocol for suspension cells.

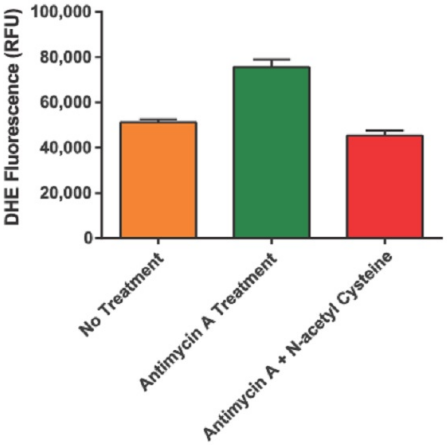

Figure 3. THP-1 cells (human monocytic leukemia cell line) 70k cells/well, treated with controls and stained using the protocol for suspension cells.

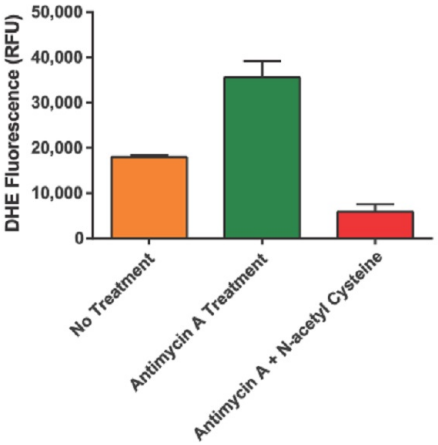

Figure 4. HepG2 cells (human liver hepatocellular carcinoma cell line) 60k cells/well, treated with controls and stained using the protocol for adherent cells.

9. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | 400 921 0189 / +86 21 2070 0500
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829