



Multidrug Resistance Assay Kit (Calcein AM)

Item No. 600370

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
400146	Cell-Based Assay Calcein AM	1 vial/50 µl	-20°C
10009322	Cell-Based Assay Buffer Tablet	1 vial/1 tablet	RT
10011234	Cell-Based Propidium Iodide Solution	1 vial/250 µl	4°C
600371	Cell-Based Assay Cyclosporin A	1 vial/50 µl	-20°C
600372	Cell-Based Assay Verapamil	1 vial/50 µl	-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.

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 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. 6-, 12-, 24-, or 96-well plates for culturing cells
2. Cells such as MES-SA/Dx5 (can be obtained from ATCC), known to over-express multidrug resistance proteins such as P-glycoprotein and MRP and the parental line, MES-SA; other cell lines that are known to express multidrug resistance proteins can also be used
3. Appropriate culture medium for the cell line used
4. Flow cytometer, fluorescence microscope, or plate reader capable of detecting calcein AM at excitation and emission wavelengths of 485 nm and 535 nm, respectively, and propidium iodide at excitation and emission wavelengths of 560 nm and 617 nm, respectively

About This Assay

Cayman's Multidrug Resistance Assay Kit provides a convenient tool for studying the modulation of cellular multidrug resistance (MDR) machinery. The kit employs calcein AM, a cell-permeable nonfluorescent dye, which is cleaved intracellularly to yield the impermeant fluorescent molecule, calcein. This calcein is rapidly excluded from cells expressing the MDR proteins P-gp or MRP, and so functions as a probe for the detection of chemical compounds inhibiting MDR proteins. Propidium iodide is provided as a stain for marking dead or damaged cells. Cyclosporin A, a competitive inhibitor of MDR proteins, and verapamil, a noncompetitive inhibitor, are included as controls.

NOTE: All the fluorescent reagents are light sensitive. Do not expose to direct intense light.

Reagent Preparation

Cell-Based Assay Buffer

Dissolve each Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

Calcein AM Staining Solution (2X)

Prepare a Calcein AM Staining Solution by adding 2 μ l of Cell-Based Assay Calcein AM (Item No. 400146) to 10 ml of the culture medium used in your experiment. Mix well and protect from light. The Calcein AM Staining Solution will be stable for one hour at room temperature. *NOTE: Protect from light.*

Propidium Iodide Solution

Prepare a staining solution by diluting the Cell-Based Propidium Iodide Solution (Item No. 10011234) 1:10 in Assay Buffer prepared above. Mix well. *NOTE: PI is a potential carcinogen. It is recommended that the user wear protective clothing, gloves, and eye/face protection in order to avoid contact with skin and eyes.*

Flow Cytometry

1. Culture cells in a 6-, 12-, or 24-well plate at a density of 5×10^5 - 2×10^6 cells/ml in a CO₂ incubator overnight at 37°C. *NOTE: Optimal conditions will depend on the cell type.*
2. On the day of the experiment, aspirate the medium from the wells and treat with culture medium containing test compounds or vehicle controls in a CO₂ incubator at 37°C. Incubation times may vary depending on the test compound. For the positive control inhibitors that are supplied in this kit (Cyclosporin A and Verapamil), a 30 minute incubation is recommended. For Cyclosporin A (Item No. 600371), dilute 1:1,000 in culture medium. For Verapamil (Item No. 600372), dilute 1:2,000 in culture medium.
3. At the end of the treatment, add an equal volume of the prepared Calcein AM Solution (2X) to each of the sample wells and incubate for an additional 30 minutes in a CO₂ incubator at 37°C.
4. Harvest the cells from each well and transfer to a tube that is compatible with your flow cytometer. Adherent cells must be dislodged by scraping or trypsin treatment to enable transfer.
5. Centrifuge the samples for five minutes at 400 x g at room temperature. Decant the supernatant from each sample being careful not disturb the cell pellet.
6. Add 1 ml of culture medium and 10 µl of diluted Propidium Iodide Solution (prepared above) to each sample. Vortex to ensure that all cells are well suspended.
7. Analyze the samples with a flow cytometer immediately. Cells taking up Calcein AM are detectable on the FITC channel while dead cells stained by propidium iodide are detectable in either the PE or PerCP channels.

Plate Reader

We recommend analysis by flow cytometry for the most accurate analysis of calcein retention. If a cytometer is not available, the following protocol can be used with a fluorescence plate reader. If following this protocol, please see the reference to the Calculations section for equations to determine the percentage of calcein retention.

1. Culture cells in a 96-well, clear-bottom, black culture plate at a density of 5×10^4 cells/well in 100 µl of culture medium. Grow cells overnight in a CO₂ incubator at 37°C. *NOTE: Optimal conditions will depend on the cell type.*
2. On the day of the experiment, centrifuge the plate for five minutes at 400 x g at room temperature.
3. Aspirate the medium from the wells and treat with 100 µl of culture medium containing test compounds or vehicle controls in a CO₂ incubator at 37°C. Incubation times may vary depending on the test compound. For the positive control inhibitors that are supplied in this kit (Cyclosporin A and Verapamil), a 30 minute incubation is recommended. For Cyclosporin A (Item No. 600371), dilute 1:1,000 in culture medium. For Verapamil (Item No. 600372), dilute 1:2,000 in culture medium. *NOTE: Differences in the number of live cells can significantly affect results. Ensure that experimental compounds used do not result in significant cell death.*

4. At the end of the treatment, add 100 μ l of the prepared Calcein AM Solution (2X) to each of the sample wells and incubate for an additional 30 minutes in a CO₂ incubator at 37°C.
5. Centrifuge the plate for five minutes at 400 x g at room temperature.
6. Aspirate the supernatant and add 200 μ l of ice-cold medium to each well, being careful to not disturb the cell layer.
7. Repeat steps 5 and 6 once more.
8. Add 200 μ l ice-cold medium to each well.
9. Analyze the samples with a fluorescent plate reader immediately. Cells taking up Calcein AM display strong fluorescence intensity with excitation and emission wavelengths of 485 nm and 535 nm, respectively.

ANALYSIS

Calculations

1. Determine the average calcein fluorescence for each sample.
2. Determine the percentage of calcein retention using the equation below.

% Calcein Retention_{Parental Cells} =

$$\left[\frac{(\text{Calcein Fluorescence of Treated Parental Cells})}{(\text{Calcein Fluorescence of Untreated Parental Cells})} \right] \times 100$$

% Calcein Retention_{MDR Cells} =

$$\left[\frac{(\text{Calcein Fluorescence of Treated MDR Cells})}{(\text{Calcein Fluorescence of Untreated Parental Cells})} \right] \times 100$$

Performance Characteristics

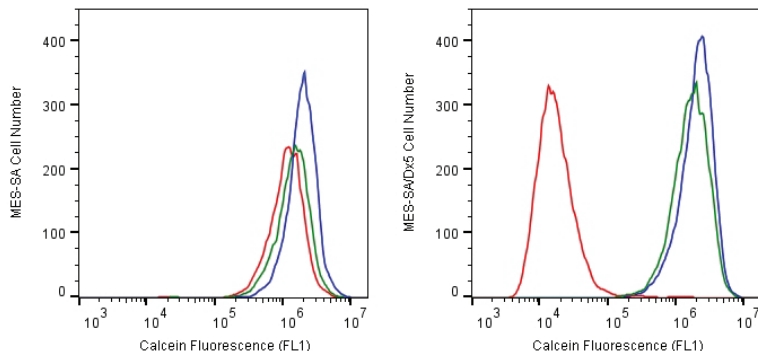


Figure 1. Both cyclosporin A and verapamil show an increase in intracellular calcein fluorescence. MES-SA and MES-SA/Dx5 cells were plated at a density of 5×10^5 cells/well and grown overnight. The following day, cells were treated with either 25 $\mu\text{g}/\text{ml}$ cyclosporin A (blue), 50 $\mu\text{g}/\text{ml}$ verapamil (green), or untreated (red) and then stained with calcein AM, according to the protocol described above. Both the cyclosporin A and the verapamil blocked the actions of the MDR transporters, causing a significant shift in intracellular calcein retention in the MDR positive cells, as evidenced by the dramatic shift in FL1 fluorescence.

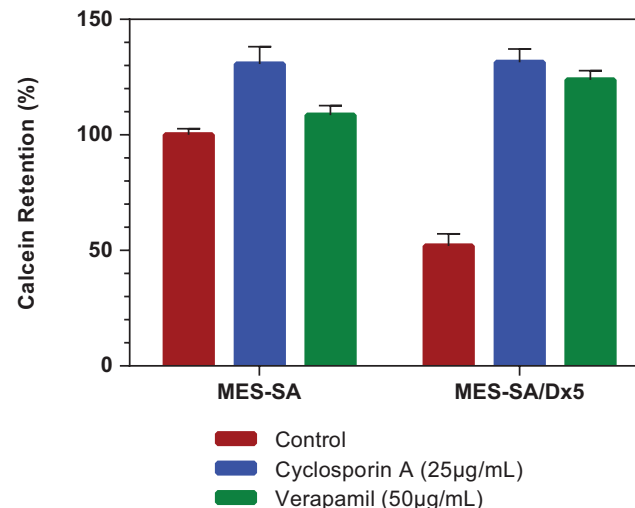


Figure 2. Cyclosporin A and verapamil increase the percentage of calcein retained within cells. MES-SA and MES-SA/Dx5 cells were plated at a density of 5×10^4 cells/well and grown overnight. The following day, cells were treated with either 25 $\mu\text{g}/\text{ml}$ cyclosporin A (blue), 50 $\mu\text{g}/\text{ml}$ verapamil (green), or untreated (red) and then stained with calcein AM, according to the protocol described above. The data were then converted to “percentage of calcein retention” using the equations above. Both the cyclosporin A and the verapamil blocked the actions of the MDR transporters, causing a demonstrable increase in intracellular calcein retention in the MDR positive cells.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Low calcein AM staining in all treatments, including positive control	Cells are not healthy	Use only healthy cells
No difference in fluorescence intensity, or strong fluorescence in all treatments	Cells are not expressing multidrug resistance proteins	Use only cells known to express multidrug resistance proteins
Low fluorescence intensity in both calcein AM and Hoechst dye staining	A. Cell density too low B. Cells lost during processing	A. Increase cell density B. Gently aspirate supernatant to ensure most of the cells stay attached to the plate

Warranty and Limitation of Remedy

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