3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Multidrug Resistance Assay Kit

Catalog Number **MAK161**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Acquired resistance to chemotherapy drugs, multidrug resistance or MDR, is a major contributor to treatment failure for many types of cancers. MDR is typically associated with the increased expression of two ATP-dependent drug efflux pumps, P-Glycoprotein (P-gp or MDR1) and the Multidrug Resistance-Associated Protein (MRP1). These pumps actively expel chemotherapeutic agents, typically hydrophobic amphipathic natural products, from the cytoplasm to outside the cell.

This kit is utilizes a hydrophobic fluorescent dye molecule to assess MDR activity in cells. This dye rapidly penetrates cell membranes and becomes trapped resulting in an increase in fluorescence intensity (λ_{ex} = 490/λ_{em} = 525 nm). In cells expressing MDR transporters, the dye is rapidly extruded by the transporters, resulting in decreased fluorescence intensity. This kit is suitable for the screening of MDR pump inhibitors or for identifying cell lines with high MDR activity.

Components

The kit is sufficient for assaying one 96 well plates (1 each) or ten 96 well plates (10 each).

Reagent	Catalog Number	1 Each	10 Each
MDR Sensor	MAK161A	1 vial	1 vial
DMSO	MAK161B	1 vial, 100 µL	1 vial, 300 µL
Assay Buffer	MAK161C	1 ea, 10 mL	1 ea, 100 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorometric assays.
- Fluorescence multiwell plate reader
- PBS or Hanks with 20 mM HEPES Buffer, pH 7.0 (HHBS)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped under ambient conditions and storage at –20 °C, protected from light, is recommended.

Procedure

Sample Preparation for One Plate

Adherent cells: Plate cells overnight in growth medium at 40,000–80,000 cells/well/90 µL for a 96 well plate or 10,000–20,000 cells/well/20 µL for a 384 well plate. Include at least 2 wells of medium only to serve as blanks.

Non-adherent cells: Centrifuge the cells from the culture medium and resuspend the cell pellets with culture medium in poly-D-lysine-coated plates at 100,000 to 200,000 cells/well/90 µL for a 96 well plate or 25,000–50,000 cells/well/20 µL for a 384 well plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiment. Include at least 2 wells of medium only to serve as blanks.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.

Assay Reaction
Allow all reagents to come to room temperature before use. Briefly centrifuge vials before opening.

1. Prepare the MDR Sensor Stock Solution by adding 20 µL (1 Each kit) or 200 µL (10 Each kit) of DMSO to the MDR sensor vial and mixing well. 20 µL of MDR Sensor Stock Solution is sufficient for one 96 well plate. Unused Stock Solution can be aliquoted and stored at –20 °C for up to one month.

2. Set up the MDR Dye Loading Solution according to the scheme in Table 1.

Table 1.
MDR Dye Loading Solution

Reagent	Volume
MDR Sensor Stock Solution	20 µL
Assay Buffer	10 mL

Note: The MDR Dye Loading Solution is enough for one plate and should be used within 2 hours of preparation. The amount of Dye Loading Solution can be scaled if necessary.

3. Treat cells with 10 µL (96 well plate) or 5 µL (384 well plate) of 10× test compounds in suitable buffer (PBS or HHBS) for desired period of time. For blank wells (no cells), add buffer only.
   Note: It is not necessary to wash cells before adding test compounds. However, if test compounds are serum sensitive, samples can be grown in serum-free medium, or growth medium and serum factors can be removed before adding compounds at 1× in 100 µL of PBS or HHBS.

4. Incubate the cells in a 5% CO_2, 37 °C incubator for the desired period of time.

5. Add 100 µL/well for 96 well plate and 25 µL/well for 384 well plate of the MDR Dye Loading Solution to each of the sample and control wells. Incubate the cells in a 5% CO_2, 37 °C incubator for 15 minutes or longer. Do not wash cells after loading.
   Note: The appropriate incubation time depends on the individual cell type and cell concentration used and should be optimized for each cell type.

6. Monitor the fluorescence intensity (λ_{ex} = 490/ λ_{em} = 525 nm).
   Note: For non-adherant cells, it is recommended to centrifuge the cell plate at 800 rpm for 2 minutes with the brake off prior to reading the fluorescence intensity.

Results
Correct for the background by subtracting the values obtained for the blank wells from the values obtained from the samples.

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