Screen Quest™ Fluorimetric MDR Assay Kit

Catalog number: 36340, 36341
Unit size: 100 Tests, 1000 Tests

Component	Storage	Amount	
		Cat No. 36340	Cat No. 36341
Component A: MDR Sensor	Freeze (<-15 °C), Minimize light exposure	1 vial, lyophilized	1 vial, lyophilized
Component B: DMSO	Freeze (<-15 °C)	1 vial (100 µL)	1 vial (300 µL)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 mL)	1 bottle (100 mL)

OVERVIEW

Tumor cell resistance to cytotoxic drugs is considered one of the major obstacles to successful chemotherapy. Some tumors are initially resistant and never respond to cytostatic drug treatment; others initially respond well but eventually regrow and become resistant. This phenomenon may result from genetic mutations induced by the administered antitumor agent, or may represent the selection of preexisting resistant cell populations in the malignant tumor. Multi-drug resistance (MDR) is a major factor in the failure of many forms of chemotherapy. In the past few years it has become widely accepted that the resistance to chemotherapy correlates with the overexpression of at least two ATP-dependent drug-efflux pumps. These cell membrane proteins, called P-glycoprotein (Pgp, MDR1), and multidrug-resistance-associated protein (MRP1) are members of the ABC transporter family. Our assay kit uses a fluorescent MDR indicator for assaying these two MDR pump activities. This hydrophobic fluorescent dye molecule rapidly penetrates cell membranes and becomes trapped in cells. Following a short incubation, the intracellular free dye concentration can increase significantly. In the MDR1 and/or MRP1-expressing cells this dye is extruded by the MDR transporter, thus decreasing the cellular fluorescence intensity. However, when its extrusion is blocked by an agent that interferes with the MDR1 and/or MRP1 pump-activity, its cellular fluorescence intensity increases significantly. Our MDR assay kit provides all the essential components with an optimized assay method. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation. This assay kit is ideal for high throughput screening of MDR pump inhibitors or identifying the cells that have high level of MDR pump activities.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Add MDR inhibitors or compounds
3. Add MDR dye-loading solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
4. Incubate at room temperature for 1 hour
5. Monitor fluorescence intensity at Ex/Em = 490/525 nm with bottom read mode

Important Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

MDR sensor stock solution:
Add 20 µL (**Cat. # 36340-1 plate**) or 200 µL (**Cat. # 36341-10 plates**) of DMSO (Component B) into MDR sensor (Component A), and mix them well.

Note 20 µL of MDR sensor stock solution is enough for one plate. Un-used MDR sensor stock solution can be aliquoted and stored at < -20 °C for one month if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles and moisture.

PREPARATION OF WORKING SOLUTION

MDR dye-loading solution:
Add 20 µL of MDR sensor stock solution into 10 mL of Assay Buffer (Component C), and mix them well.

Note The MDR dye-loading solution is enough for one plate and stable for at least 2 hours at room temperature.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells with test compounds by adding 10 µL of 10X (96-well plate) or 5 µL of 5X (384-well plate) compounds into compound buffer (such as PBS or HHBS). For blank wells (medium without the cells), add the corresponding amount of compound buffer.

   Note It is not necessary to wash cells before adding compound. However, if tested compounds are serum sensitive, growth medium and serum factors can be aspirated away before adding compounds. Add the same volume of HHBS into the wells (such as 90 µL for a 96-well plate or 20 µL for a 384-well plate) after aspiration. Alternatively, cells can be grown in serum-free media.

2. Incubate the cell plate at room temperature or in a 37 °C, 5% CO_2 incubator for at least 15 minutes or a desired period of time.

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of MDR dye-loading solution.

4. Incubate the dye-loading plate at room temperature for 1 hour, protected from light.

   Note The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment. (We got the optimal results with the incubation time less than 4 hours.)

   Note DO NOT wash the cells after loading.

   Note For non-adherent cells, it is recommended to centrifuge the cell plate at 800 rpm for 2 minutes with brake off after incubation.

5. Monitor the fluorescence intensity at Ex/Em = 490/525 nm with bottom read mode.

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EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Cyclosporin samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

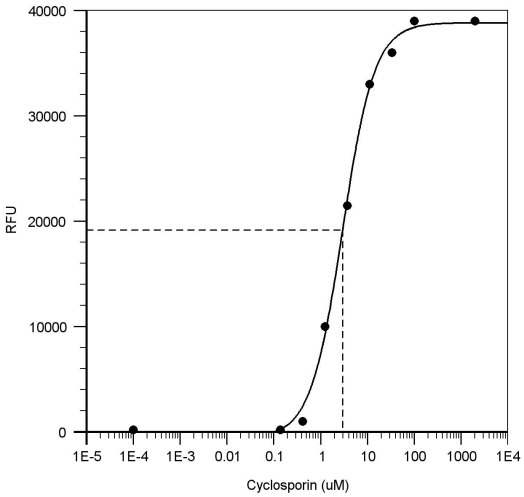

Figure 1. Effect of Cyclosporin A on the inhibition of P-gp pump in MCF-7/ADR cells. The increased concentration of Cyclosporin A resulted in an increase in fluorescence signal caused by the inhibition of P-gp pump which enhanced the intracellular accumulation of MDR indicator dye. The EC50 = 2.4 μM (measured with the kit) is similar to the value reported in the literature.

DISCLAIMER