Screen Quest™ Fluorimetric MDR Assay Kit

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 36340(1 plate), 36341(10 plates)	Keep in freezer and protect from light	All fluorescence bottom-reading microplate readers

Introduction

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 Screen Quest™ Fluorimetric MDR Assay Kits use 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 transporters, 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. The Screen Quest™ Fluorimetric MDR Assay Kits are ideal for high throughput screening of MDR pump inhibitors or identifying the cells that have high level of MDR pump activities.

Kit Components

Components	**Amount**	
	Cat. # 36340 (1 plate)	**Cat. # 36341 (10 plates)**
Component A: MDR Sensor	1 vial, lyophilized	1 vial, lyophilized
Component B: DMSO	1 vial (100 µL)	1 vial (300 µL)
Component C: Assay Buffer	1 bottle (10 mL)	1 bottle (100 mL)

Materials Required (but not provided)

- A 96 or 384-well microplate: A tissue culture microplate with black wall and clear bottom.
- A Fluorescence microplate reader with a filter set of Ex/Em = 490/525 nm, and bottom read mode.
- HHBS (1X Hank's with 20 mM Hepes Buffer, pH 7.0) or PBS.

Assay Protocol for One 96-well Plate

Brief Summary

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

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 40,000 to 80,000 cells/well/90 µL for a 96-well plate or 10,000 to 20,000 cells/well/20 µL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in culture medium at 100,000-200,000 cells/well/90 µL for a 96-well poly-D lysine plate or 25,000-50,000 cells/well/20 µL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments. *Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density.*

2. Prepare dye-loading solution:

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

2.2 Make 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.

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2.3 Make MDR dye-loading solution for one cell plate: Add 20 µL of MDR sensor stock solution (from Step 2.2) into 10 mL of Assay Buffer (Component C), and mix them well. The MDR dye-loading solution is stable for at least 2 hours at room temperature.

3. Run MDR assay:

3.1 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.

3.2 Incubate the cell plate at room temperature or in a 37 ^{o}C, 5% CO_2 incubator for at least 15 minutes or a desired period of time.

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

3.4 Incubate the dye-loading plate at room temperature for 1 hour, protected from light. (The incubation time could be from 15 min to overnight. We got the optimal results with the incubation time less than 4 hours.)
Note 1: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.
Note 2: DO NOT wash the cells after loading.
Note 3: For non-adherent cells, it is recommended to centrifuge the cell plate at 800 rpm for 2 minutes with brake off after incubation.

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

Data Analysis

The fluorescence in blank wells added with the growth medium is subtracted from the values for those wells with cells treated with the test compounds. The background fluorescence of the blank wells may vary depending on the sources of the microtiter plate or the growth media.

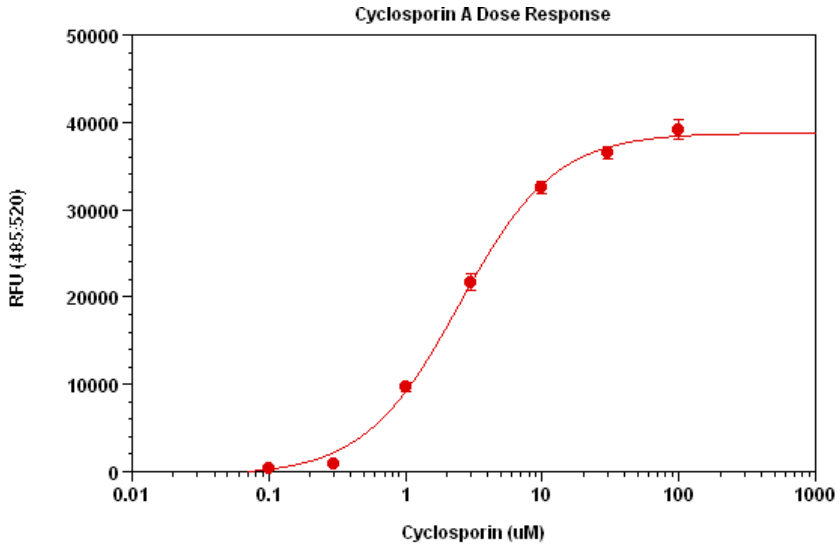

Figure 1. Effect of Cyclosporin A on the inhibition of P-gp pump in MCF-7/ADR cells**.** The increased concentration of Cyclosporin A resulted in an increase in fluorescence signal caused by the inhibition of P-gp pump which enhanced the intracellular accumulation of MDR indicator dye. The EC_{50} = 2.4 µM (measured with the kit) is similar to the value reported in the literature.