

ab112142

MDR Assay Kit (Fluorometric)

Instructions for Use

For detecting MDR pump activities in cells using our proprietary fluorescence probe

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

Version 2 Last Updated 8 October 2013

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1. 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.

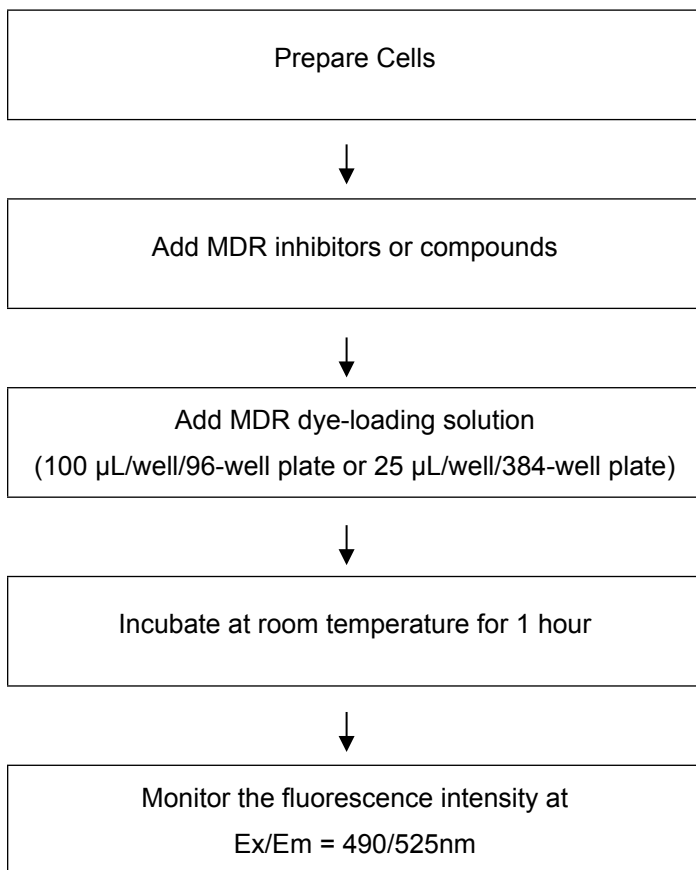
ab112142 use a fluorescent MDR indicator for assaying these two MDR pump activities. This hydrophobic fluorescent dye molecule rapidly penetrates cell membranes and becomes trapped in cells. Following a short incubation, the intracellular free dye concentration can increase significantly. In the MDR1 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. ab112142 is ideal for high throughput screening of MDR pump inhibitors or identifying the cells that have high level of MDR pump activities.

Kit Key Features

- **Increased Signal Intensity:** Higher maximum signal with lower variance across the plate
- **Flexible Dye Loading:** Dye loading at RT instead of 37 °C for 30 minutes to 1 hour.
- **Convenient:** Formulated to have minimal hands-on time. No wash step needed.
- **Versatile Applications:** Compatible with many cell lines and targets.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	1 x 96 tests	10 x 96 tests
MDR Sensor (Lyophilized)	1 vial	1 vial
DMSO	1 x 100 μ L	1 x 300 μ L
Assay Buffer	1 x 10 mL	1 x 100 mL

4. Storage and Handling

Keep at -20°C. Avoid exposure to light.

5. Additional Materials Required

- 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.
- HHBS (1X Hank's with 20 mM Hepes Buffer, pH 7.0) or PBS.

6. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of Cells

1. For adherent cells: Plate cells overnight in growth medium at 40,000 to 80,000 cells/well/90 μ L for a 96-well plate or 10,000 to 20,000 cells/well/20 μ L for a 384-well plate.
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 μ L for a 96-well poly-D lysine plate or 25,000-50,000 cells/well/20 μ L 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.

B. Preparation of Dye-loading Solution

1. Thaw all the kit components at room temperature before use.
2. Make MDR sensor stock solution: Add 20 μL or 200 μL of DMSO into MDR sensor 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 $< -20\text{ }^{\circ}\text{C}$ for one month if the tubes are sealed tightly. Protect from light and avoid repeated freeze-thaw cycles.

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

C. Run MDR Assay:

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. (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.

5. Monitor the fluorescence intensity at
Ex/Em = 490/525 nm.

7. 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.

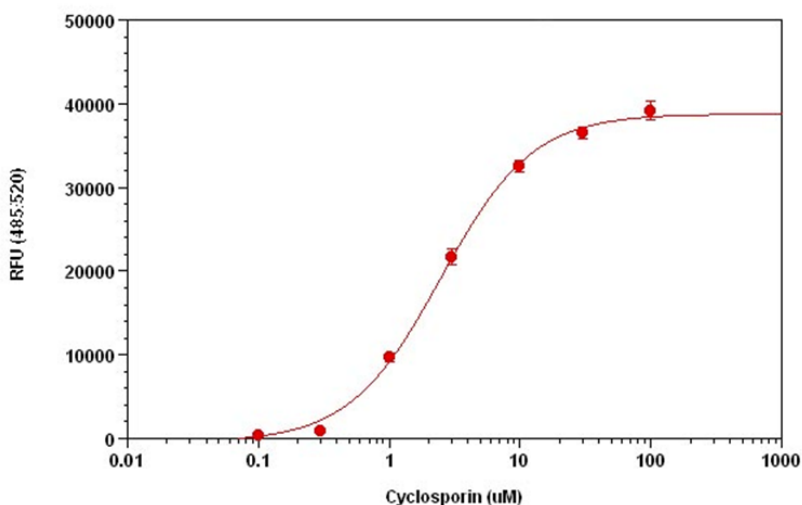


Figure 1. Effect of Cyclosporin A on the inhibition of P-gp pump in MCF-7/ADR cells. The increased concentration of Cyclosporin A resulted in an increase in fluorescence signal caused by the inhibition of P-gp pump which enhanced the intracellular accumulation of MDR indicator dye. The $EC_{50} = 2.4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

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the 10kDa spin column (ab93349) or Deproteinizing sample preparation kit (ab93299)
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

UK, EU and ROW

Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria

Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France

Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany

Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain

Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland

Email: technical@abcam.com

Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America

Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada

Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific

Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan

Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp