# ab284553 – Multidrug Efflux Transporter P Glycoprotein (MDR1/P-gp) Ligand Screening Kit

For the screening of screening test compounds for modulation of efflux transporter activity in MDR1-expressing cell lines.
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:**
http://www.abcam.com/ab284553

## Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Efflux Assay Buffer | 50 mL | -20°C |
| Fluorogenic P-gp Substrate | 1 vial | -20°C |
| P-gp Inhibitor (Verapamil) | 1 vial | -20°C |

## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Cell line for testing: cells with high levels of endogenous P Glycoprotein (e.g. A549/ADR, KB-V1 or MES-SA/MX2 drug-resistant cancer cell lines) or heterologous cells stably transfected with human MDR1 (e.g. MDCKII-MDR1 cells) are recommended.
- 1 (e.g. MDCKII-MDR1 cells) are recommended.
- Multi-well fluorescence microplate reader.
- Precision multi-channel pipette and reagent reservoir.
- Sterile anhydrous (reagent grade) DMSO.
- White-walled 96-well plates with clear flat bottom.

## Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Efflux Assay Buffer: Allow to thaw to room temperature under sterile conditions. Store at 4°C.

Fluorogenic P-gp Substrate: Reconstitute with 55 µl anhydrous DMSO and vortex thoroughly to obtain a 400X stock solution. Aliquot the stock solution as desired and store aliquots at -20°C, protected from light. Avoid repeated freeze/thaw cycles

P-gp Inhibitor (Verapamil): Reconstitute with 110 µl anhydrous DMSO and vortex until fully dissolved to obtain a 100X stock solution. Store at -20°C, stable for at least 4 freeze/thaw cycles.

## Assay Protocol

### Multidrug Efflux Transporter (MDR1/P-gp) Ligand Screening Protocol

The procedure described below is for a 96-well plate format but may be adapted to other formats by scaling the reagent volumes and cell density according to the desired plate size. To ensure assay consistency, we recommend that each treatment condition (including no inhibition and maximal inhibition controls) be performed in duplicate or triplicate wells.

Cell Culture and Seeding:

1. If using an **adherent cell line**, split cells one day prior to assay and seed approximately 3-5 x $10^4$ cells/well in a white-walled 96-well plate (with clear bottom) using 200 µl appropriate culture media/well. Grow cells overnight in a 5% $CO_2$ atmosphere 37°C incubator (cell monolayer should be ≈80-90% confluent for optimal assay).
2. **For suspension cell lines**, pellet cells by centrifugation, replace standard growth medium with serum-free, phenol red-free medium and plate approximately 1-2 x $10^5$ cells/well using 100 µl medium/well in a white-walled, clear bottom 96-well plate.

**Efflux Assay Reaction and Test Compound Preparation:**

1. Pre-warm Efflux Assay Buffer to 37°C. For adherent cells, gently aspirate culture medium, wash the cells once with 100 µl Efflux Assay Buffer to ensure complete removal of medium and add 100 µl fresh Efflux Assay Buffer to each well before returning cells to incubator. For suspension cells, no wash step is needed if cells have been plated in 100 µl serum-free, phenol red-free medium/well.
2. Dissolve test compounds in proper solvent(s) to produce stock solutions. For each test compound, prepare a 4X solution of each desired test concentration by diluting stock solutions in Efflux Assay Buffer. To determine $IC_{50}$ values for test compounds, 4X test compound solutions should be prepared in a range of concentrations in order to generate a multi-point dose-response curve (the concentration of organic solvent should be the same for all test compound dilutions). Final organic solvent concentration should be minimized to avoid impacting cell health or P-gp efflux pump activity. We recommend that the 4X test compound solutions contain 4% DMSO (1% final concentration), as DMSO has been shown to have little effect on P-gp activity at a concentration of ≤2% (v/v).
3. Prepare a 4X maximal inhibition control solution by adding 20 µl of the 100X verapamil stock to 480 µl Efflux Assay Buffer. The maximal inhibition control (Verapamil, 100 µM final concentration) serves as a definition of 100% inhibition of P-gp mediated efflux. Prepare a 4X control solution (no inhibition) by adding 20 µl of anhydrous DMSO to 480 µl Efflux Assay Buffer (1% DMSO at final concentration). Add 50 µl of either 4X test compound solution, 4X maximal inhibition control solution or 4X no inhibition control solution to each well. Each plate should contain its own maximal inhibition and no inhibition control wells.
4. Prepare 4X solution of Fluorogenic P-gp Substrate by adding 50 µl of the 400X stock solution to 4950 µl pre-warmed Efflux Assay Buffer. This preparation is sufficient for 100 reaction wells, but can be scaled depending upon the number of reactions to be performed. The 4X Fluorogenic P-gp Substrate working solution should be made fresh prior to use.
5. Add 50 µl of 4X Fluorogenic P-gp Substrate to each well (for a final reaction volume of 200 µl per well) and incubate the plate at 37°C in a 5% $CO_2$ atmosphere, protected from light for 30 min.

## Measurement

After 30 min incubation, measure the fluorescence intensity (Ex/Em = 488/532 nm) of all of the wells in end-point mode using the 'bottom read' function of the spectrofluorometer

## Calculation

1. For each test compound (TC) well, quantify the relative inhibition of P-gp mediated substrate efflux using the equation below, where $F_{vehicle}$ is the fluorescence intensity of the no inhibition control condition (solvent control), $F_{max}$ is the defined maximal inhibition control condition (100 µM verapamil) and $F_{TC}$ is the fluorescence intensity of a test compound at the given concentration:

$$\% \text{ Activity} = 100 - \left( \frac{F_{TC} - F_{vehicle}}{F_{max} - F_{vehicle}} \times 100 \right)$$

## Technical Support

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