EFLUXX-ID® Green multidrug resistance assay kit

Simple No-wash Assay for Simultaneous Monitoring of All 3 Major ABC Transporter Proteins – MDR, BCRP & MRP

The EFLUXX-ID® Multidrug Resistance Assay Kit allows for functional detection of all three clinically relevant ABC transporter proteins: MDR1 (p-glycoprotein), MRP1/2, and BCRP. The assay uses a hydrophobic, non-fluorescent compound that readily penetrates the cell membrane, where it is hydrolyzed to a hydrophilic fluorescent dye by intracellular esterases. Unless the EFLUXX-ID® dye is pumped out of the cell, the esterase cleaved dye is trapped inside the cell. Thus, cells exhibiting drug resistance will have diminished fluorescence. EFLUXX-ID® assay is the only available kit for the simultaneous monitoring of all three major ABC reporter proteins with the ability to profile individual pump activity.

Mechanism of Action

The proprietary AM-ester form of the EFLUXX-ID® dye is a hydrophobic non-fluorescent compound that readily penetrates the cell membrane and is subsequently hydrolyzed inside of the cells by intracellular esterases. Unless the EFLUXX-ID® dye is pumped out of the cell, the esterase cleaved dye is trapped inside the cell. The fluorescence signal of the dye generated within the cells thus depends upon the activity of the ABC transporters. The cells with highly active transporters will demonstrate lower fluorescence because of the active efflux of the probe from the cell. Application of specific inhibitors of the various ABC transporter proteins allows differentiation between the three common types of pumps.

Drug resistance is a phenomenon mediated by up-regulation of a family of transmembrane ATP Binding Cassette (ABC) transporter proteins. Overexpression of ABC transporter proteins accelerates removal of toxic agents from the cell, for example the efflux of chemotherapeutic agents from tumor cells, or of antibiotics from resistant strains of bacteria.

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Ordering Information

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ENZ-51029-K100	1Kit

- Measures drug resistance related to activity of all three major ABC transporter proteins.
- Single proprietary dye provides quantitative measurements of MDR activity in live cells expressed as MDR Activity Factor (MAF).
- Kit includes three known inhibitors specific for MDR1 (p-glycoprotein), MRP1/2, and BCRP.
- Simple, no-wash protocol delivers results in 1 hour.
- Available in green and gold fluorophores.
- Comprehensive efflux detection assay for three major types of ABC transporters.
- Detects BCRP activity not detected with Calcein AM.
- Inhibitors included for profiling of specific pumps involved in drug resistance.
- Two dye formats allows multiplexing with GFP-expressing cell lines or other CELLESTIAL® dyes.

Manuals, SDS & CofA

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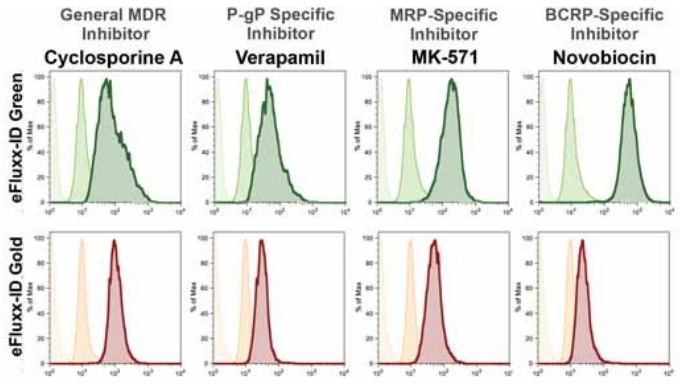


Figure 2: Profiling of ABC transporter activity by known inhibitors was assessed in CHO K1 cells using eFluxx-ID® Green and eFluxx-ID® Gold dyes. Cells were incubated for 5 min at 37°C with general MDR Inhibitor (far left column) or transporter-specific inhibitors included in the kit. Cells were then loaded with the indicated dye for 30 min at 37°C and immediately analyzed by flow cytometry. Inhibitors used: 5 µM Cyclosporin A (general MDR inhibitor), 20 µM Verapamil (specific P-gp inhibitor); 0.05 mM MK-571 (specific MRP inhibitor), 0.05 mM Novobiocin (specific BCRP inhibitor).

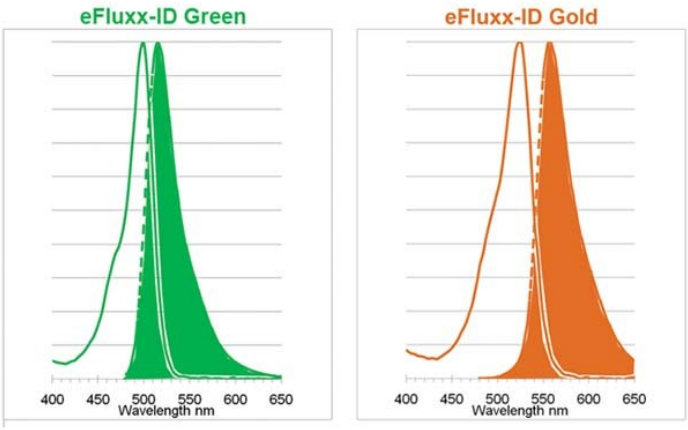
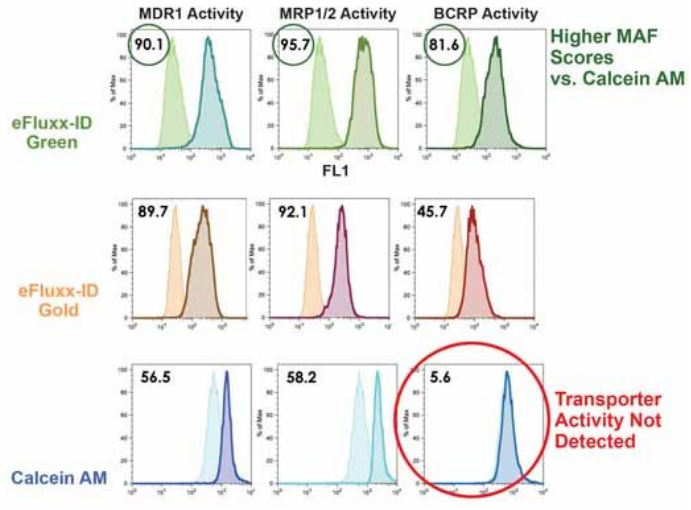


Figure 3: Spectral characteristics of the eFluxx-ID Green 490/514 nm ex/em and Gold 530/570 nm ex/em reagents allows for multiplexing with other common fluorescent dyes.



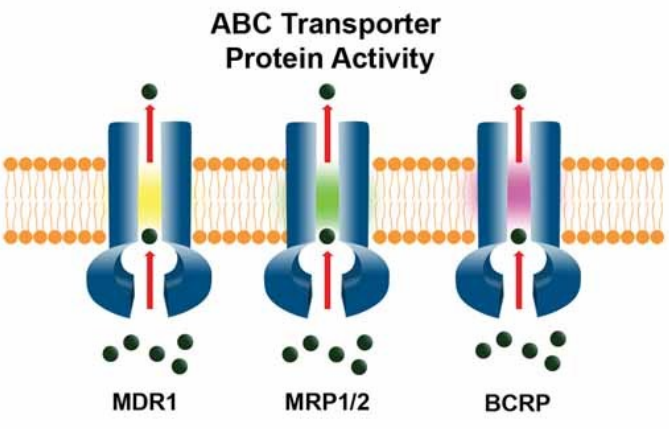


Figure 1: Detect activity of all three major ABC transporter proteins. ABC transporter protein activity was evaluated in CHO K1 cells by flow cytometry using eFluxx-ID® Green (top), Gold (middle), or Calcein AM (bottom) dyes. Treatment with specific inhibitors of ABC Transporter proteins (shaded histograms) induces retention of dye within cells relative to untreated cells (lined histograms). The difference in mean fluorescence intensity (MFI) is an indication of the corresponding protein activity, as shown by MAF scores [multidrug resistance activity factors], a quantitative measurement of multidrug resistance. Higher MAF scores are a result of superior specificity of eFluxx-ID dyes to specific inhibitors. Calcein AM (a common probe for MDR assays), is unable to detect BCRP activity.

Fluorophore	Compatible eFluxx-ID Reagent
Conjugates of Cy 3 & Cy 5, PI, 7-AAD, APC, Texas Red, RFP, YFP, TAMRA, UV and Violet dyes	eFluxx-ID Green
GFP/eGFP, RFP, PI, 7-AAD, APC, Conjugate of Cy 5, Rhodamine 123, Texas Red, UV and Violet dyes	eFluxx-ID Gold

Table 1: Compatibility of eFluxx-ID MDR dyes with other fluorescent dyes.

Handling & Storage

Use/Stability	With proper storage, the kit components are stable for one year upon receipt.
Handling	Protect from light. Avoid freeze/thaw cycles.
Short Term Storage	-20°C
Long Term Storage	-80°C
Shipping	Blue Ice

Regulatory Status

RUO - Research Use Only

Product Details

Application	Flow Cytometry, Fluorescence microscopy
Application Notes	The EFLUXX-ID® Green multidrug resistance assay kit is designed for functional detection and profiling of multidrug resistant phenotypes in live cells (both suspension and adherent).
Contents	1 vial, EFLUXX-ID® Green Detection Reagent 300 nmoles, MDR1 Inhibitor (Verapamil) 750 nmoles, MRP Inhibitor (MK-571) 1.5 µmoles, BCRP Inhibitor (Novobiocin) 500 µl, Propidium Iodide
Quality Control	A sample kit from each lot of EFLUXX-ID® Green multidrug resistance assay kit is used to stain CHO K1 cell line which is used between passages 5 and 20, using the procedures described in the user manual. The following results were obtained: 1. MAF >60 after verapamil- or MK-571-mediated inhibition 2. MAF >30 after novobiocin-mediated inhibition
Quantity	100 assays. For flow cytometry.

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