DATA SHEET for MRP/BCRP Vesicle Uptake Assay Reagents

MRP/BCRP ProVesicle Uptake Assay Reagents

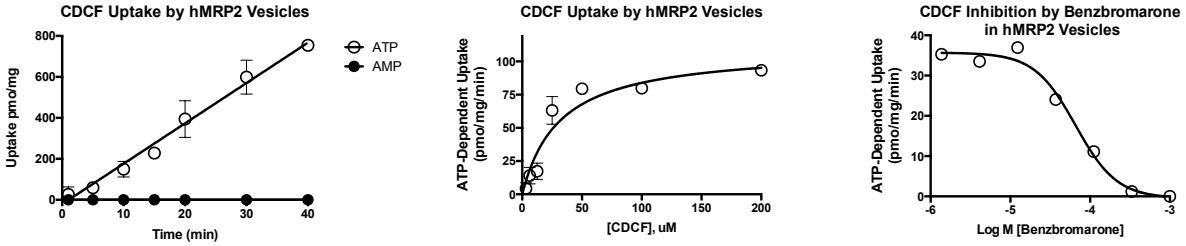
Cat. #PB21002; Lot #PN-B-1001

Contents: ***Kit contains reagents for 200 assays***

A. MRP/BCRP Uptake Buffer: 10 mM Tris-HCl, pH 7.4; 250 mM sucrose, 10 mM $MgCl_2$
B. 10 x Wash Buffer: 400 mM MOPS-Tris, pH 7.0; 700 mM KCl
C. 300 mM GSH
D. 200 mM ATP
E. 200 mM AMP

Store at 4C upon receipt.

Representative data using PB21002 showing time dependence, concentration dependence and competitive inhibition of uptake at hMRP2 when using CDCF, a fluorescent substrate:



ATP-dependent CDCF (10 µM) uptake: 40 pmol/mg/min
IC_{50} for reference inhibitor benzbromarone: 65 µM

Vesicle Uptake Assay Protocol:

1. Incubate a 95 µL reaction containing 50 µg vesicles and CDCF in MRP/BCRP Uptake Buffer (10 mM Tris-HCl, pH 7.4; 250 mM sucrose, 10 mM $MgCl_2$ and 3 mM GSH) for 5 min at 37C.
2. Uptake was started by adding MgATP (or AMP) at a final of 5 mM and incubating for 20 min at 37C.
3. Reaction was terminated by transferring vesicle samples to a filter plate and washing the filter plate 6 x with ice cold washing buffer (40 mM MOPS-Tris, pH 7.0; 70 mM KCl).
4. Filter plate was dried and fluorescent counts measured with a spectrophotometer.

Calculating ATP-Dependent Transport:

ATP-dependent transport = uptake in the presence of ATP – uptake in the presence of AMP.

This product is strictly for laboratory research use only.

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