DATA SHEET for hMRP4, *ABCC4*, ProVesicles

hMRP4 Sf9-membrane derived vesicles for uptake

Cat. #PV31004; Lot #PN-V-1001

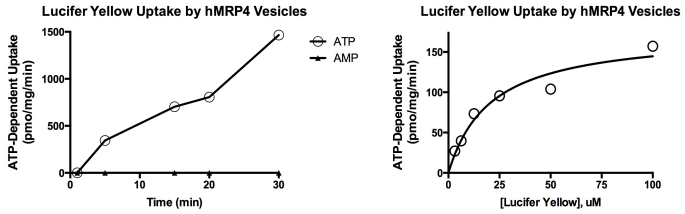
Contents: 500 µL @ 5 mg/mL total protein (determined by BCA protein assay) in MRP Resuspension Buffer*

*MRP Resuspension Buffer: 50 mM Tris-HCl, pH 7.0; 50 mM mannitol; 2 mM EGTA; 2 mM DTT; 8 µg/mL aprotinin and 10 µg/mL leupeptin.

Store at -70 to -80C upon receipt. Aliquot to smaller working volumes to minimize freeze-thawing cycles.

Human multidrug resistance protein 4 (hMRP4) inside-out vesicles are prepared from Sf9 insect cells infected with baculovirus to overexpress MRP4. ProNovus hMRP4 ProVesicles should be used to investigate drug interactions with hMRP4 *in vitro*.

Representative data showing time dependence, concentration dependence and competitive inhibition of uptake at hMRP4 when using Lucifer Yellow (LY), a fluorescent substrate:



ATP-dependent Lucifer Yellow (10 µM) uptake: 50 pmol/mg/min

Vesicle Uptake Assay Protocol:

1. Incubate a 95 µL reaction containing 50 µg vesicles and LY in MRP 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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