

DATA SHEET for hMRP2, ABCC2, ProVesicles

hMRP2 Sf9-membrane derived vesicles for uptake

Cat. #PV31003; Lot #PN-V-1005

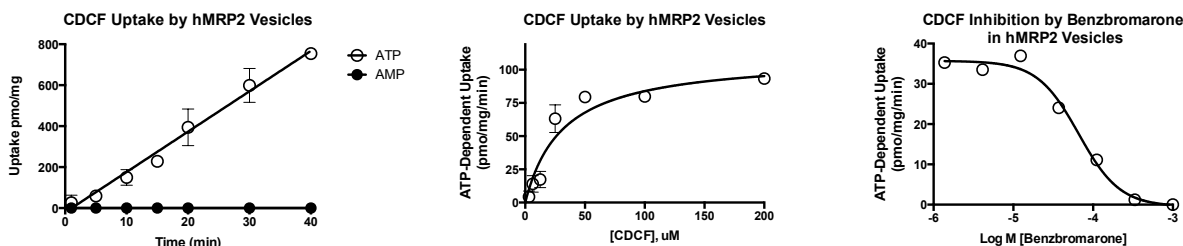
Contents: 500 μ L @ 5 mg/mL total protein (determined by BCA protein assay) in MRP2 Resuspension Buffer*

*MRP2 Resuspension Buffer: 50 mM Tris-HCl, pH 7.0; 50 mM mannitol; 2 mM EGTA; 2 mM DTT; 8 μ g/mL aprotinin and 10 μ g/mL leupeptin.

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

Human multidrug resistance protein 2 (hMRP2) inside-out vesicles are prepared from Sf9 insect cells infected with baculovirus to overexpress MRP2. ProNovus hMRP2 ProVesicles should be used to investigate drug interactions with hMRP2 *in vitro*.

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



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

Vesicle Uptake Assay Protocol:

1. Incubate a 95 μ L reaction containing 50 μ g vesicles and CDCF in MRP2 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.

