DATA SHEET for hBSEP, *ABCB11*, ProVesicles

hBSEP Sf9-membrane derived vesicles for uptake

Cat. #PV31001; Lot #PN-V-1005

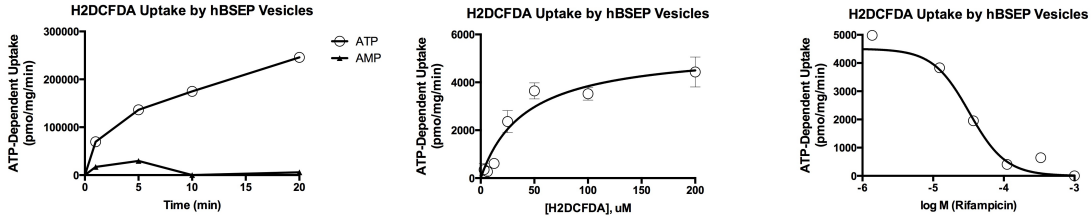
Contents: 500 µL @ 5 mg/mL total protein (determined by BCA protein assay) in BSEP Resuspension Buffer*

*BSEP Resuspension Buffer: 50 mM HEPES-Tris, pH 7.0; 50 mM sucrose; 100 mM KNO_3; 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 bile salt export pump (hBSEP) inside-out vesicles are prepared from Sf9 insect cells infected with baculovirus to overexpress BSEP. ProNovus hBSEP ProVesicles should be used to investigate drug interactions with hBSEP *in vitro*.

Representative data showing time dependence, concentration dependence and competitive inhibition of uptake at hBSEP when using 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA), a fluorescent substrate:



ATP-dependent H_2DCFDA (10 µM) uptake: 1000 pmol/mg/min
IC_{50} for reference inhibitor rifampicin: 30 µM

Vesicle Uptake Assay Protocol:

1. Incubate a 95 µL reaction containing 50 µg vesicles and H_2DCFDA in BSEP Uptake Buffer (10 mM HEPES, pH 7.5; 50 mM sucrose, 100 mM KNO_3; 12.5 mM $Mg(NO_3)_2$) 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 (10 mM Tris-HCl, pH 7.4; 100 mM KNO_3; 50 mM sucrose).
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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