DATA SHEET for BSEP Vesicle Uptake Assay Reagents

BSEP ProVesicle Uptake Assay Reagents

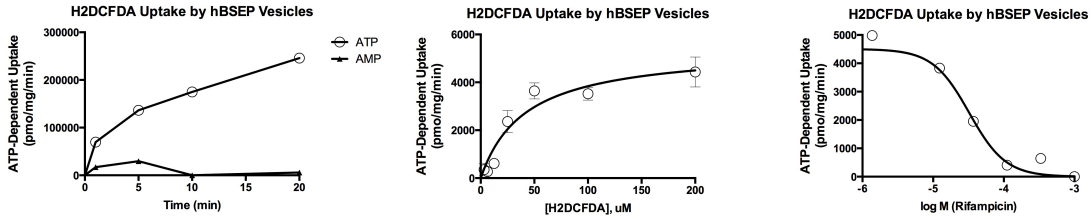
Cat. #PB21001; Lot #PN-B-1001

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

A. BSEP Uptake Buffer: 10 mM HEPES, pH 7.5; 50 mM sucrose, 100 mM KNO_3; 12.5 mM $Mg(NO_3)_2$
B. 10 x Wash Buffer: 100 mM Tris-HCl, pH 7.4; 1 M KNO_3; 500 mM sucrose
C. 200 mM ATP
D. 200 mM AMP

Store at 4C upon receipt.

Representative data using PB21001 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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