Discovery Life Sciences
6 Henshaw Street
Woburn, MA 01801
Tel: (866) 838-2798

info@dls.com
https://www.dls.com

Gentest™ BSEP Vesicle Assay Kit
Data Sheet

Catalog Number:……459011 **Date Released:** ……...2024 August
Lot Number:……2402190 **Expiration Date:** …… 2026 February
Storage Conditions: STORE AT -20°C (Individual components can be stored as instructed below)

Uptake Assay Results:

- Uptake Activity in the presence of ATP: pmol/(mg membrane protein) x (min)
- Uptake Activity in the presence of AMP: pmol/(mg membrane protein) x (min)
- Uptake assay was performed using all the components in the current kit lot and the indicated batch of transporter vesicles.

Transporter	Batch #	Probe Substrate	Final Conc.	Uptake Activity in the presence of ATP	Uptake Activity in the presence of AMP
Human BSEP (453802)	2302108	Taurocholic Acid	1 µM	72	4

Kit Components

1. **Assay Uptake Buffer** Volume: 20 mL Storage Condition: 2-8°C or below Buffer Composition: 10 mM Hepes-Tris pH 7.4, 100 mM KNO_3, 12.5 mM $Mg(NO_3)_2$, 50 mM Sucrose	2. **10X Wash Buffer** Volume: 2 X 30 mL Storage Condition: 2-8°C or below Buffer Composition: 100 mM Hepes-Tris pH 7.4 , 1M KNO_3, 500 mM Sucrose
3. **200 mM ATP:** Adenosine 5'-Triphosphate, Magnesium in H_2O, pH 7.0 Volume: 2 X 250 µL Storage Condition: -20°C	4. **200 mM AMP:** Adenosine 5′-monophosphate disodium in H_2O, pH 7.0 Volume: 2 X 250 µL Storage Condition: -20°C
5. **1mM Taurocholic Acid:** in DMSO, Positive control for BSEP Volume: 100 µL Storage Condition: -20°C Solvent: DMSO	6. **Blocking Buffer (50X):** 5% Bovine Serum Albumin. Volume: 1.0 mL Storage Condition: -20°C

Note

- Freeze-thaw stability: This vesicle assay kit may be subjected to 6 freeze-thaw cycles without a change in uptake activity or a significant loss of signal-to-noise ratio for the included positive control.

Discovery Life Sciences
6 Henshaw Street
Woburn, MA 01801
Tel: (866) 838-2798

info@dls.com
https://www.dls.com

- The Gentest BSEP vesicle assay kit is designed to characterize the substrates or inhibitors of Bile Salt Export Pump (BSEP). The assay is based on measuring the cumulative uptake of test compounds in the inside-out vesicles, prepared from Human Embryonic Kidney (HEK) 293 cells infected with a recombinant baculovirus encoding the cDNA for varied ABC transporters, e.g. BSEP. BSEP is an important hepatobiliary transporter and inhibition of BSEP can lead to hepatic cholestasis in patients. This model can be applied to directly determine BSEP substrate and inhibitor specificity. The instructions are for conducting screening and inhibition assays using radioisotope-labeled compounds. The assay kit provides sufficient reagents for 200 assays (4 vials of vesicle products). These instructions can accommodate screening assays for 30 test compounds and 1 positive control; or conduct an IC_{50} assay for 7 test compounds and 1 positive control
- TransportoCells™ HEK293-derived ABC Transporter Membrane Vesicles instructions for use describes how to perform the vesicle uptake assay using the TransportoCells™ ABC transporter vesicle products listed below.

Kit Components/Storage Conditions

Name	Description	Quantity	Storage Temperature
Assay Uptake buffer	Hepes Uptake Buffer (10 mM Hepes-Tris pH 7.4, 100 mM KNO_3, 12.5 mM $Mg(NO_3)_2$, 50 mM Sucrose)	20 mL	≤ 4°C
10x Wash buffer	10X Hepes Wash Buffer (100 mM Hepes-Tris pH 7.4, 1M KNO_3, 500 mM Sucrose)	50 mL	≤ 4°C
200 mM ATP	Adenosine 5'-triphophate, Magnesium Salt	500 µL	≤ -20°C
200 mM AMP	Adenosine 5′-monophosphate disodium salt	500 µL	≤ -20°C
1 mM Taurocholic Acid	TCA, hBSEP substrate	100 µL	≤ -20°C
Block buffer	5% Bovine Serum Albumin	100 µL	≤ -20°C

Reagents and Disposables Not Supplied in Kit

Reagent/Disposable	Vendor	Catalog Number
Deionized Water (dH2O)	-	-
Dimethyl Sulfoxide (DMSO)	Millipore Sigma	D2438
Reagent Reservoirs	Corning	RES-V-50-SI
1.7 mL Microcentrifuge tubes	Corning	MCT-175-C-S
[^{3}H] Taurocholic Acid	Perkin Elmer	NET322
96-well Black Flat-Bottom Assay Microplate	Corning	3915
96-well Clear Assay Microplate	Corning	353075
96-well Glass Fiber (G/F) Filter Microplate	Perkin Elmer	6005177
96-well Glass Fiber (G/F) Filter Microplate	Millipore Sigma	MSFBN6B10
Plate Seals	Perkin Elmer	6005185
Betaplate Scintillation Liquid	Perkin Elmer	1205-440

Discovery Life Sciences
6 Henshaw Street
Woburn, MA 01801
Tel: (866) 838-2798

info@dls.com

https://www.dls.com

Recommended Materials and Equipment

Orbital Shaker
Centrifuge
37°C Incubator
Water Bath
Ice Bucket
Wet Ice
Scintillation Counter or Equivalent
Fluorescence Microplate Reader
LC-MS/MS
Cell Harvester or Vacuum Manifold
Multichannel Pipetman and Corresponding Tips

Gentest ABC Transporter Vesicles	Catalog Number	Gene Accession Number
Control Vesicles	453800	N/A
Human BSEP Vesicles	453802	NM_003742

Safety Recommendations:
Safety assessment indicates this product is hazardous; refer to SDS [Safety Data Sheet] for safety information. Handle in accordance with good industrial hygiene and laboratory safety practices.

Quality Assurance

23 August 2024

Date

Discovery Life Sciences
6 Henshaw Street
Woburn, MA 01801
Tel: (866) 838-2798

info@dls.com

https://www.dls.com

Appendix A: TROUBLESHOOTING GUIDE

Trouble	Guide
1. Test samples are not showing any activity	• Test compounds may not be substrates for the transporter protein expressed in the membranes. • The test concentration may be too low. Increase the test compound concentration or increase the concentration of radiolabeled test compounds. • Make sure the final solvent (DMSO, ethanol, methanol) concentration is lower than 2%. • Check the purity of the radiolabeled test compounds. • Decay of radioactivity for certain radiolabeled compounds can affect the detection level for radioactivity. • Recommend use of low binding glass vial to prepare the reaction mixture if the test compounds show high binding affinity to plastic.
2. Positive controls are showing lower activity or no activity	• TCA demonstrates high binding on GF/B filter plate. Make sure to apply blocking buffer on the filter plate before harvesting reaction. • When the cell harvester from Perkin-Elmer is used, make sure the orientation of the plate is appropriate (upside of reaction plate is at downside of GF/B filter plate). • The stock concentration of radiolabeled substrates varies batch to batch. Make sure the calculation of radiolabeled substrate is correct. • Be sure to prepare the proper dilutions of vesicles and ATP as described in this manual. • Make sure the wash buffer is chilled before use (2°C to 8°C). • Make sure the filter plate is completely dry before further processing. Activity and signal-to-noise ratio may be lower if not dried properly. • Do not vortex vesicle/substrate mix. Vigorous vortexing may destroy the vesicular structure, resulting in low activity or low signal-to-noise ratio.
3. High background is observed with test compounds	• Recommend running a test compound alone (w/o vesicles) to test for non-specific binding of the test compound on GF/B filter plates. • If high-binding affinity is observed for the test compound on GF/B filter plates, pre-blocking the filter with a low concentration of 0.1% bovine serum albumin or high concentration of cold test compound may reduce the background effectively.
4. Triplicate values are inconsistent with each other	• It is important to be accurate with pipetting. Due to the sensitivity of the assay, slight changes in pipetting (using different pipets, tips, etc.) could result in inconsistent values. • Make sure the rapid filtration system functions properly, and is washed thoroughly before starting the assay. Blocked channels or residual radioactivity on the equipment can cause variation in the assay.