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ProductInformation

BSEP, human
recombinant, expressed in *Sf*9 cells,
membrane preparation, for Vesicular Transport

Product Code **B2436**
Storage Temperature –70 °C

TECHNICAL BULLETIN

Product Description

The bile salt export pump (BSEP/ABCB11) belongs to the family of ATP-binding-cassette (ABC) transporters and has also been called the sister of P-glycoprotein (sister Pgp).[1] Most ABC transporters transport substrates across the cell membrane using ATP as an energy source. BSEP is the major bile salt transporter in the liver canalicular membrane and it is inhibited by a number of drugs or drug metabolites. This is potentially a significant mechanism for drug-induced cholestasis.[2] Dysfunction of individual bile salt transporters such as BSEP, due to genetic mutation, suppression of gene expression, disturbed signaling, or steric inhibition, is an important cause of cholestatic liver disease.[3]

One of the simplest methods invented for measuring transport is the vesicular transport assay. The human BSEP transporter is expressed in *Sf*9 insect cells using the baculoviral expression system. Membrane preparations from infected cells always contain some closed membrane vesicles that have an inside-out orientation (5-10% of total lipid). In the case of these inside-out vesicles, transport of substrates across the membrane takes molecules from the surrounding buffer and transports them into the vesicles. The rate of this transport is temperature and ATP dependent.

The procedure in this kit determines the interaction of compounds with the BSEP transporter using the vesicular transport assay. The interaction is detected by changes in the initial rate of ^{3}H-taurocholic acid transport by BSEP into membrane vesicles purified from *Sf*9 cells expressing the transporters.

Rapid filtration of the membrane suspension through a filter that retains membrane vesicles allows removal of the substrate molecules that are “outside” leaving the membrane vesicles with transported molecules trapped “inside” on the filter.

The quantity of transported molecules can be determined by any adequate method like HPLC, LC/MS/MS separation and detection. Also, the transported molecules can be labeled with fluorescent or radioactive tags. This protocol utilizes ^{3}H-taurocholic acid for the detection of the transported substrate in a competition type assay.

BSEP mediates the transport of taurocholic acid (TC) very efficiently. Compounds that interact with the transporter modulate the initial rate of TC transport measured without any other compounds added. If a substance is a transported substrate of the transporter it might compete with TC, thus reducing the rate of TC transport. If a compound is an inhibitor of the transporter, it will block the transport of TC into the membrane vesicles. Some compounds can be co-transported with TC. These substances will increase the rate of TC transport compared to the control level.

Reagent

The membrane vesicles are suspended in 50 mM Hepes-Tris with 100 mM KNO_3 and 50 mM sucrose, pH 7.4.

Equipment and Reagents Required But Not Provided

- HEPES, Product Code H4034
- Trizma® base [Tris(hydroxymethyl)aminomethane, Tris-base], Product Code T1503
- Potassium nitrate (KNO_3), Product Code 221295
- Magnesium nitrate, hexahydrate [$Mg(NO_3)_2 \cdot 6H_2O$], Product Code M5296
- Sucrose, Product Code S0389
- Adenosine 5′-triphosphate, disodium salt (ATP), Product Code A2383
- Cyclosporin A, Product Code C3662
- Sodium taurocholate, Product Code T4009
- Dimethyl sulfoxide (DMSO), Product Code D2650

- ^{3}H-Taurocholic acid, ~1 mCi/ml
- OptiPhase SuperMix scintillation cocktail, PerkinElmer Product Code 1200-439
- Ultrapure water (17 MΩ·cm or equivalent)
- Filter plates (Millipore MultiScreen®$_{HTS}$ 96 well Filter Plates with glass fiber filter or equivalent, Millipore Product Code MSFB N6B 10)
- Rapid filtration apparatus (Millipore 96 well plate filtration system or equivalent)
- Plate incubator/shaker
- Multichannel pipettes with corresponding tips
- 96 well liquid scintillation system

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Use ultrapure water (17 MΩ·cm or equivalent) for preparation of reagents.

1.7 M Tris Solution - Dissolve 20.587 g of Tris-base in 100 ml of water. The solution may be stored at 2–8 °C for at least 1 year.

10 mM HEPES-Tris, pH 7.4 Solution – Dissolve 0.24 g of HEPES in 80 ml of water. Adjust pH to 7.4 with 1.7 M Tris Solution. Bring final volume to 100 ml with water. The solution may be stored at 2–8 °C for at least 1 year.

100 mM Tris-HCl, pH 7.4 Solution – Dissolve 1.21 g of Tris-base in 80 ml of water. Adjust pH to 7.4 with 5-10 M HCl solution. Bring final volume to 100 ml with water. The solution may be stored at 2–8 °C for at least 1 year.

1 M KNO_3 Solution – Prepare in water. The solution may be stored at 2–8 °C for at least 1 year.

0.1 M $Mg(NO_3)_2$ Solution – Prepare in water. The solution may be stored at 2–8 °C for at least 1 year.

1.5 mM Cyclosporin A Solution – Prepare in DMSO. The solution may be stored at –20 °C for at least 1 year.

0.2 M Mg-ATP Solution - Dissolve 2.2 g of ATP and 0.813 g of $MgCl_2$ in 10 ml of water and adjust pH to 7.0 with 1.7 M Tris Solution. Bring final volume to 20 ml with water. The solution may be stored at –20 °C for at least 1 year.

200 µM Sodium Taurocholate Solution – Prepare in water. The solution may be stored at –20 °C for at least 1 year.

Assay Mix – Combine the following:

10 mM HEPES-Tris, pH 7.4 Solution	2 ml
1 M KNO_3 Solution	1 ml
0.1 M $Mg(NO_3)_2$ Solution	1 ml
water	6 ml

Dissolve 0.171 g of sucrose in the mixture and sterile filter. The solution can be stored at 2–8 °C.

Washing Mix – Combine the following:

100 mM Tris-HCl, pH 7.4 Solution	50 ml
1 M KNO_3 Solution	50 ml
water	400 ml

This incomplete solution can be prepared and stored at 2–8 °C. Prior to use, dissolve 8.6 g of sucrose and 0.027 g of sodium taurocholate in this solution to give the complete Washing Mix.

Alternatively, the complete Washing Mix with sucrose and sodium taurocholate can be prepared, sterile filtered, and stored at 2–8 °C.

Storage/Stability
Store the product at –70 °C.

Procedure
This procedure is for the determination of the interaction of compounds with the BSEP transporter using the ^{3}H-taurocholate vesicular transport assay in a 96 well format.

Positive control – Cyclosporin A inhibits the ^{3}H-TC transport of the BSEP transporter. Due to this inhibition, it can be used as a positive control by replacing the test compound with 1 µl of 1.5 mM Cyclosporin A Solution giving a final concentration 20 µM. Controls should be run in duplicate with both Assay Mix and Assay Mix with ATP.

Membrane negative control – There is a low endogenous ^{3}H-TC transport detected in membranes expressing a mutant (defective) variant of the MRP1 transporter (Product Code M9819; MDR1, MRP, and BSEP Control). However, for the study of transport of cold compounds, use of this control as a negative control is suggested.

96 Well Assay

1. Prepare the Reaction Suspension – Combine the following:

Membrane Suspension	1,000 µl
^{3}H-Taurocholic acid, ~1 mCi/ml	10 µl
200 µM Sodium Taurocholate Solution	x* µl
Assay Mixture	3,990 – x* µl

*The amount of unlabeled 200 µM Sodium Taurocholate Solution to add depends on the concentration of the labeled ^{3}H-TC used. This concentration can be calculated from the information supplied with the labeled ^{3}H-TC. Add the volume of the unlabeled 200 µM Sodium Taurocholate Solution required for a final total (labeled and unlabeled together) TC concentration of 2 µM in the Reaction Suspension.

2. Add 50 µl of the Reaction Suspension to the wells of a standard 96 well plate, **not** the filter plate.

3. Add test compound solutions to give the final reaction concentrations indicated in Table 1. The volume of the test compound solution should not exceed 1.5 µl. Add DMSO to the solvent only assays (row H).
Note: If the test compound is not dissolved in DMSO, substitute the solvent used in the wells marked for DMSO.

4. Prepare Assay Mix with ATP – Add 90 µl of the 0.2 M Mg-ATP Solution to 1,410 µl of Assay Mix.

5. Preincubate the plate, 1,500 µl of the Assay Mix, and 1,500 µl of the Assay Mix with ATP at 37 °C for 5 minutes.

6. Wet the filter plate and set up the filtration apparatus.

7. Add 25 µl of Assay Mix with ATP or Assay Mix to the wells as indicated in Table 1. Shake plate with the shaker. Incubate the plate at 37 °C for 5 minutes.
Note: Depending on the equipment available, the assays can be run one row at a time or in blocks. The general consideration is that the filtration should take place within 2 minutes after stopping the assay with cold Assay Mix (step 8).

8. Stop the reaction by adding 200 µl of ice cold Washing Mix to each well. Transfer the reaction mixtures to the filter plate and filter.

9. Wash each well five times, each time with 200 µl of ice cold Washing Mix.

10. Pipette 2.5 µl the Reaction Suspension (prepared in step 1) into one well of the filter plate. The radioactivity (cpm) measured on this filter in this well is the total activity per well/20 for each assay.

11. Dry the filter plate. A heat gun (hair drier) may be used to speed up the process.

12. Add 50 µl of scintillation cocktail and measure radioactivity in each well. Record cpm values.

Table 1.
Assay Layout Guidelines - Preparation of reaction mixtures

	1	2	3	4	5	6	7	8	9	10	11	12
	Compound 1				**Compound 2**				**Compound 3**			
	Assay Mix with ATP		**Assay Mix (without ATP)**		**Assay Mix with ATP**		**Assay Mix (without ATP)**		**Assay Mix with ATP**		**Assay Mix (without ATP)**	
A	100 µM		100 µM		100 µM		100 µM		100 µM		100 µM	
B	33.3 µM		33.3 µM		33.3 µM		33.3 µM		33.3 µM		33.3 µM	
C	11.1 µM		11.1 µM		11.1 µM		11.1 µM		11.1 µM		11.1 µM	
D	3.7 µM		3.7 µM		3.7 µM		3.7 µM		3.7 µM		3.7 µM	
E	1.23 µM		1.23 µM		1.23 µM		1.23 µM		1.23 µM		1.23 µM	
F	0.41 µM		0.41 µM		0.41 µM		0.41 µM		0.41 µM		0.41 µM	
G	0.137 µM		0.137 µM		0.137 µM		0.137 µM		0.137 µM		0.137 µM	
H	DMSO		DMSO		DMSO		DMSO		DMSO		DMSO	

Calculations

ATP dependent transport (cpm) – For each tested compound, subtract cpm values measured without the presence of ATP (Assay Mix) from the cpm values measured in the presence of ATP (Assay Mix with ATP) for controls and samples. Take the average of the duplicates.

ATP dependent transport (pmol/mg/min) – Multiply the total activity per well/20 by 20 to get Total activity (cpm). Calculate the rate of transport in pmol/mg membrane protein/min using the following:

$$\frac{\text{ATP dependent transport (cpm)}}{\text{Total activity (cpm)}} \times \frac{\text{TC concentration (nM)} \times \text{Volume (ml)}}{\text{membrane protein (mg)} \times \text{time (minutes)}}$$

For assays performed according to the described procedure, the value of the right part of the equation is 600. This calculation uses 0.075 ml as the reaction volume and excludes the small variable volume of the added test compound solution.

ATP dependent transport (%) – Calculate the percent activation or inhibition of the test compound. In this representation the ATP dependent transport determined in the compound free (solvent only) control is taken as 100% and all other values are represented on this relative scale. Use the following formula:

$$\frac{\text{ATP dependent transport in the presence of test compound (cpm)}}{\text{ATP dependent transport in compound free control (cpm)}} \times 100$$

References

1. Childs, S. *et al*., Identification of a sister gene to P-glycoprotein. Cancer Res., **55**, 2029-34 (1995).
2. Byrne, J.A. *et al*., The human bile salt export pump: Characterization of substrate specificity and identification of inhibitors. Gastroenterology, **123**, 1649-58 (2002).
3. Kullak-Ublick, G.A. *et al*., Enterohepatic bile salt transporters in normal physiology and liver disease. Gastroenterology, **126**, 322-42 (2004).

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