Discovery Life Sciences
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Gentest™ MRP/BCRP Vesicle Assay Kit

Catalog Number:……459010 **Date Released:** 2024 August
Lot Number:……2402281 **Expiration Date:** 2026 February
Storage Conditions: STORE AT -80°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 membrane.

Transporter	Batch #	Probe Substrate	Final Conc.	Uptake Activity in the presence of ATP*	Uptake Activity in the presence of AMP*
Human MRP3 (453805)	2210316	Estradiol-17β-glucuronide	1 µM	22	2.0
Human BCRP (453804)	2164001	Estrone-3-Sulfate	1 µM	110	6.8

Kit Components

1. **Assay Uptake Buffer** Volume: 20 mL Storage Condition: 2-8°C or below Buffer Composition: 47 mM MOPs, 65 mM KCl, 7 mM $MgCl_2$, pH 7.4	2. **10X Wash Buffer** Volume: 2 X 25 mL Storage Condition: 2-8°C or below Buffer Composition: 400 mM MOPs, 700 mM KCl, pH 7.4
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. **300 mM GSH:** L-Glutathione in H_2O, pH 6.8 Volume: 0.1 mL Storage Condition: -20°C	6. **20 mM Estradiol-17B-Gluc:** Estradiol-17β-glucuronide, Positive Control for MRP2/MRP3 Volume: 50 µL Storage Condition: -20°C Solvent: DMSO
7. **1 mM Estrone-3-sulfate:** in DMSO, Positive Control for BCRP Volume: 100 µL Storage Condition: -20°C Solvent: DMSO	8. **100 µM LTC4:** Leukotriene C4 in DMSO, Positive Control Volume: 100 µL Storage Condition: -80°C Solvent: DMSO
9. **1 mM CDCF:** 5(6)-Carboxy-2,'7'-Dichlorofluorescein, fluorescent substrate for MRP2 Volume: 100 µL Storage Condition: -20°C Solvent: DMSO	

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Notes

- 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 controls.
- The Gentest™ MRP/BCRP vesicle assay kit is designed to characterize the substrates or inhibitors of MRP/BCRP transporters. 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 efflux transporters, e.g. MRPs and BCRP. This model can be applied to directly determine the substrates or inhibitors of ABC transporters. The assay kit provides sufficient reagents for 200 assays and requires 4 vials of vesicles.
- 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 and Storage Conditions

Name	Description	Quantity	Storage Temperature
Assay Uptake buffer	MOPs Uptake Buffer (47 mM MOPs, 65 mM KCl, 7 mM $MgCl_2$, pH 7.4)	20 mL	≤ 4°C
10x Wash buffer	10X MOPs Wash Buffer (400 mM MOPs, 700 mM KCl, pH 7.4)	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
300 mM L-Glutathione	GSH, Co-factor	100 µL	≤-20°C
20 mM Estradiol-17β-Gluc	Estradiol-17β-glucuronide, MRP2/MRP3 substrate	50 µL	≤-20°C
1 mM Estrone-3-sulfate	BCRP substrate	100 µL	≤-20°C
1 mM CDCF	Fluorescent substrate, 5(6)-Carboxy-2,'7'-Dichlorofluorescein, MRP2 substrate	100 µL	≤-20°C
100 µM Leukotriene C4	LTC4	100 µL	≤-80°C

Reagents and Disposables Not Supplied in Kit

Reagent/Disposable	Vendor	Catalog Number
Deionized Water (dH2O)	-	-
Dimethyl Sulfoxide (DMSO)	MilliporeSigma	D2438
Reagent Reservoirs	Corning	RES-V-50-SI
1.7 mL Microcentrifuge tubes	Corning	MCT-175-C-S
0.1N Sodium hydroxide (NaOH)	J.T.Baker	5636-02
0.1N Sulfuric Acid (H_2SO_4)	MilliporeSigma	319589
Sodium Dodecyl Sulfate (SDS)	MilliporeSigma	L3771
[^{3}H] Leukotriene C4	Perkin Elmer	NET1018
[^{3}H] Estradiol-17β-D-glucuronide	Perkin Elmer	NET1106
[^{3}H] Estrone-3-sulfate	Perkin Elmer	NET203
[^{3}H] Dehydroepiandrosterone	Perkin Elmer	NET860
Dehydroepiandrosterone	Sigma	D-065
N-methyl-quinidine	MIlliporeSigma	SBNMQ
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	MilliporeSigma	MSFBN6B10
Plate Seals	Perkin Elmer	6005185
Betaplate Scintillation Liquid	Perkin Elmer	1205-440

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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
Pipets-10, 20, 200, 1000µL Pipets and Corresponding Tips

Gentest ABC Transporter Vesicles	Catalog No.	Gene Accession No.
Human MDR1/P-gp Vesicles	453801	NM_000927
Human MRP2 Vesicles	453803	NM_000392
Human BCRP Vesicles	453804	NM_004827
Human MRP3 Vesicles	453805	NM_003786
Human MRP4 Vesicles	453806	NM_005845

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

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Appendix A: TROUBLESHOOTING GUIDE

1. Test samples do not show 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. In general, radioactivity should be greater than 0.2 µCi/well. • 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 show lower activity or no activity.	• 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 substrates 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 (2-8°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 mixture. 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 bovine serum albumin or high concentration of cold test compounds 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 out thoroughly before starting the assay. Blocked channels or residual radioactivity on the equipment can cause variation in the assay.