

Rat Brain Endothelial Cell (RBE) Monolayer SERVICE

The rat brain endothelial cell (RBE) monolayer assay is an excellent in vitro test system to model the Blood-Brain-Barrier (BBB) and study the brain penetration of compounds by evaluating their vectorial transport.

General Features

- suitable for studying the blood-brain-barrier penetration of compounds: „permeability studies”
- in vitro assay
- toxicity test: 5 concentrations of TA in triplicate

Specific Features

- brain endothelial cell co-culture system with pericytes and astrocytes (more details on Page 4.)
- Mdr1a transporter specific setups are available: Mdr1a inhibitor assessment (Drug-drug Interaction) and Mdr1a substrate assessment
- substrates and inhibitors are harmonized with SOLVO’s rat brain microdialysis services

Check out SOLVO’s comprehensive blood-brain-barrier transporter testing solutions on our homepage.

SOLVO is developing its **Bcrp1-specific rat brain endothelial cell (RBE) monolayer assay**. For further information, please, contact us!

Studies available

1. Permeability studies
2. Mdr1a substrate assessment studies
3. Mdr1a inhibitor assessment (DDI) studies

Setups available

SOLVO also offers two setups for the above studies, which differ in the amount of information derived and costs.

1. Basic setup - Studies at one concentration
2. Extended setup - Studies at several concentrations, more controls

Study parameters can be customized to suite your needs. The following tables summarize some adjustable parameters.

Optional parameters for RBEC monolayer studies

Parameter	Options	Effect on Price
Unidirectional	A-B or B-A	depends on nr of wells
Nr. of replicates	triplicates or more	depends on nr of wells
TA concentration	no limitations	no effect on price
Nr of TA concentrations	no limitations	depends on nr of wells
Sink conditions	+ 1% BSA in receptor compartment	no effect on price
Non-specific binding	wells without cells	depends on nr of wells
Nr. of samples taken	time points and compartments	depends on nr of LC/MS/MS analytical samples

1. Permeability studies (uni- and bi-directional)

		Basic	Extended
assay conditions	concentration TA	1	3
	samples (minutes)	R: 0, 15, 30, 60	R: 0, 15, 30, 60
	R:receptor D:donor	D: 0, 60	D: 0, 60
	Nr of replicates	3	3
	Direction	BA or AB/BA	BA or AB/BA
controls	non-specific binding	wells without cells	wells without cells
	permeability	Sodium fluorescein	Sodium fluorescein
		Evans blue albumin	Evans blue albumin
	functionality	quinidine, AB, BA	quinidine, AB, BA
	monolayer confluency	TEER	TEER
toxicity	5 concentrations of TA	5 concentrations of TA	

2. Mdr1a substrate assessment studies

		Basic	Extended
assay conditions	concentration TA	1	2
	samples (minutes)	R: 0, 60	R: 0, 60
	R:receptor D:donor	D: 0, 60	D: 0, 60
	specific inhibitor for Mdr1a	PSC833	PSC833
	Nr of replicates	3	3
	Direction	AB/BA	AB/BA
controls	non-specific binding	wells without cells	wells without cells
	permeability	Sodium fluorescein	Sodium fluorescein
		Evans blue albumin	Evans blue albumin
	functionality	quinidine, AB, BA	quinidine, AB, BA
	specific inhibitor for Mdr1a	PSC833	PSC833
	monolayer confluency	TEER	TEER
toxicity	5 concentrations of TA	5 concentrations of TA	

3. Mdr1a inhibitor assessment (Drug-drug Interaction) studies

		Basic	Extended
assay conditions	concentration TA	1	5
	samples (minutes)	R: 0, 60	R: 0, 60
	R:receptor D:donor	D: 0, 60	D: 0, 60
	probe substrate	3H-quinidine	3H-quinidine
	Nr of replicates	3	3
	Direction	AB/BA	AB/BA
controls	non-specific binding	wells without cells	wells without cells
	permeability	Sodium fluorescein	Sodium fluorescein
		Evans blue albumin	Evans blue albumin
	functionality	quinidine, AB, BA	quinidine, AB, BA
	toxicity	5 concentrations of TA	5 concentrations of TA
	specific inhibitor for Mdr1a	PSC833	PSC833
	monolayer confluency	TEER	TEER

Validation data

Moreover, the contribution of the Mdr1a transporter to the active absorption/efflux processes can be determined by the application of selective substrate (quinidine) and inhibitor (PSC833) (Figure 1.).

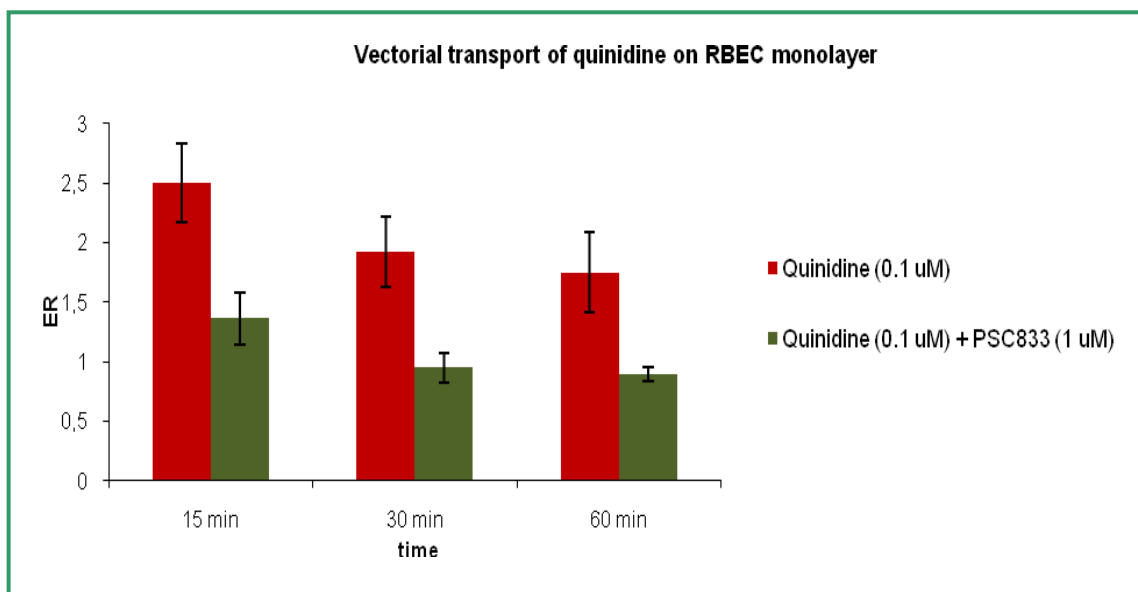


Figure 1. Transport of Quinidine via RBEC monolayer in the absence or presence of Mdr1a inhibitor, PSC833 at various time points

Scientific background

SOLVO's RBEC (Rat Brain Endothelial Cell) monolayer assay applies primary rat brain microvascular endothelial cells isolated from two-week old rats, co-cultured with rat pericytes and rat astrocytes (triple co-culture system).

Endothelial cells are cultured on the upper side of a semipermeable porous filter, having pericytes on the backside and astrocytes in the bottom of the wells of the culture plate (Figure 2.). Brain endothelial cells in contact with pericytes and astrocytic factors form a tight monolayer which separates two solute compartments, above and below the cell layer, that corresponds to the capillary lumen (apical side) and the brain interstitium (basolateral side), respectively.

Under appropriate cell culture conditions, RBEC monolayers maintain the main characteristics of the cerebral endothelium *in vivo* such as expression of von Willebrand factor, high activity and inducibility by astrocytes of the alkaline phosphatase and gamma glutamyl transpeptidase activity.

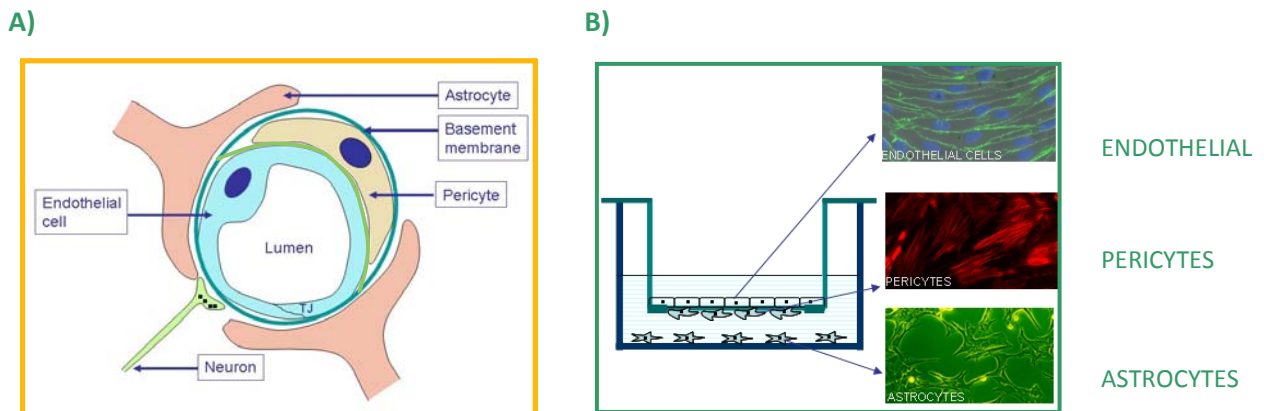


Figure 2. In vivo (A) and in vitro (B) representation of the Blood-brain-barrier