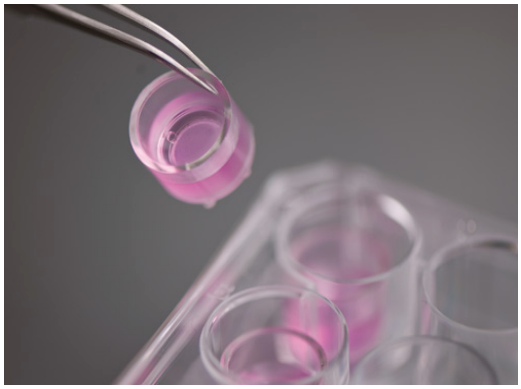
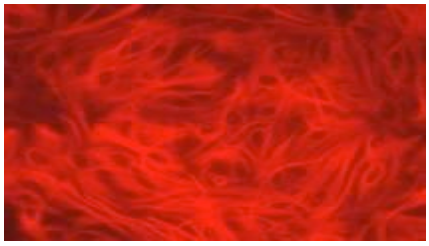
# 3D Human Blood Brain Barrier Model

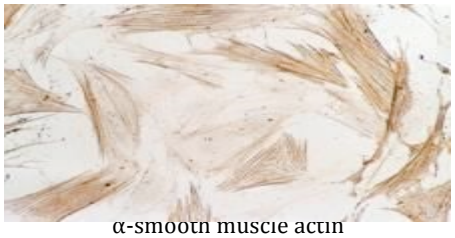
| | |
|---|---|
| **Product Name** | 3D Human Blood Brain Barrier |
| **Catalog Number** | EP010 |
| **Product Format** | 6 , 12, and 24 well |
| **Storage Temperature** | -80°C. |
| **Shipping Temperature** | -80°C. |



**CNS pharmaceutics market:** Only a few central nervous system (CNS) disorders, such as depression, epilepsy, chronic pain, and affective disorders, respond to clinical treatments by the 2% of small-molecule drugs that we have; on the other hand, many more serious CNS disorders cannot be effectively treated by these small therapeutic molecules including Alzheimer disease, Huntington disease, stroke, brain cancer, brain and spinal cord injury, HIV infection of the brain, therefore we want to help change that by creating our **3DHuman BBB model**. The blood brain barrier (BBB) specifically regulates molecular and cellular flux between the blood and the nervous tissue. We develop and characterize a highly reproducible Human in *vitro* model of the BBB using co-cultures of primary Human brain endothelial cells (HBEC), Human brain pericytes, and Human brain astrocytes to study receptors involved in transcytosis across the endothelial cell monolayer. Many drugs developed to treat Central Nervous System (CNS) disorders are unable to reach the brain parenchyma in therapeutically relevant concentrations. The BBB protects brain nervous tissue from the fluctuation of plasma composition, from pathogenic agents, and maintains homeostasis of the brain parenchyma by restricting non-specific flux of ions, peptides, proteins and even cells into and out the brain.



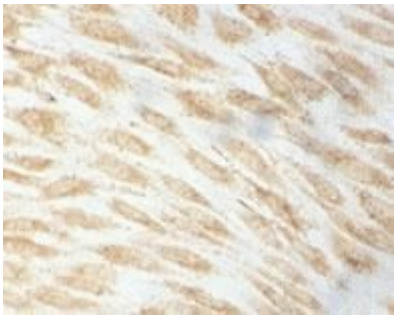
GFAP



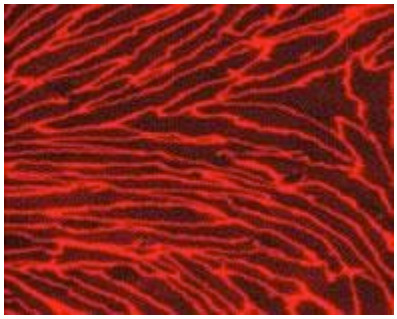
α-smooth muscle actin

Fig.1. Expression of GFAP in primary Human astrocytes and α-smooth muscle actin in primary Human pericytes

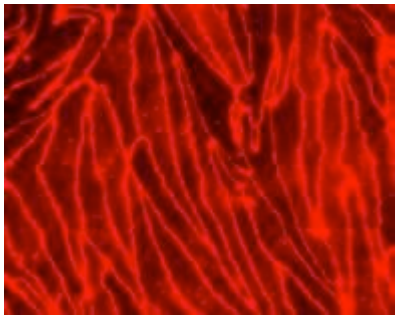
Astrocytes and brain pericytes help to develop and maintain specific BBB characteristics in brain capillary endothelial cells. Co-culture of the three cell types in our 3D Human BBB model led to the enhancement of barrier properties; an increase in expressions of tight junction proteins of occludin, claudin-5 and ZO-1 and continuous localizations of ZO-1 and claudin-5.



Factor VIII



ZO-1



claudin-5

Fig.2. cells are immunopositive for the astrocytes marker glial fibrillary acidic protein (GFAP), while the remaining 10 % is immunopositive for CD11b, a marker of microglia.

Our model mimic transport properties of the BBB due to the formation of tight junctions, higher expression of specific carriers, or great cell viability. We developed a 3D in vitro model of the BBB by culturing brain endothelial cells with pericytes and astrocytes layered

in an insert. **This model improves endothelial cell polarization and enhance the formation of tight junctions, provide better endothelial cell-to-cell contact that is important for barrier development, and prevent the dilution of secreted neurotrophic factors, and these conditions collectively led to the development of an in vitro model that can truly mimic the BBB.**

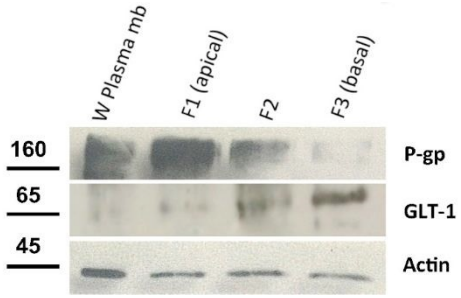
## Advantages:

1) Cells used in the 3D model are all human cells; results obtained are more relevant to human situations rather than those data from animal models, i.e. CAM et al.
2) The whole process can be monitored (from cell inoculation to the end of experiment), therefore, more crucial information can be acquired at multiple time points from a single experiment.
3) No need to perform post-experimental staining for endothelial markers, this is particularly important, if those markers are changed in experimental conditions involved in the studies.

The 3D Human BBB Model contains all the materials necessary to perform multiple angiogenesis assays in 6, 12, or 24 well formats.

**The 3D Human BBB model can be use, but not limited to:**

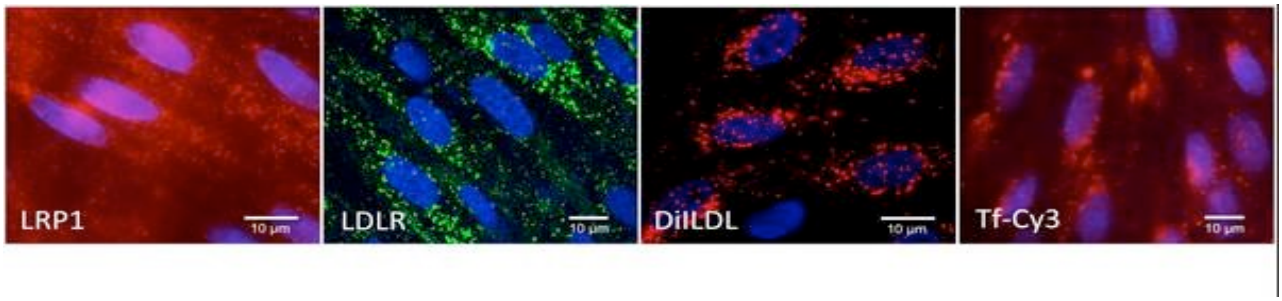
- Drug BBB permeability assay
- Research on BBB physiology
- Cell-cell interactions
- Transport pathway modulations
- Research on BBB toxicology
- Brain endothelial toxicity assays
- Research on BBB pathology
- Disease modeling



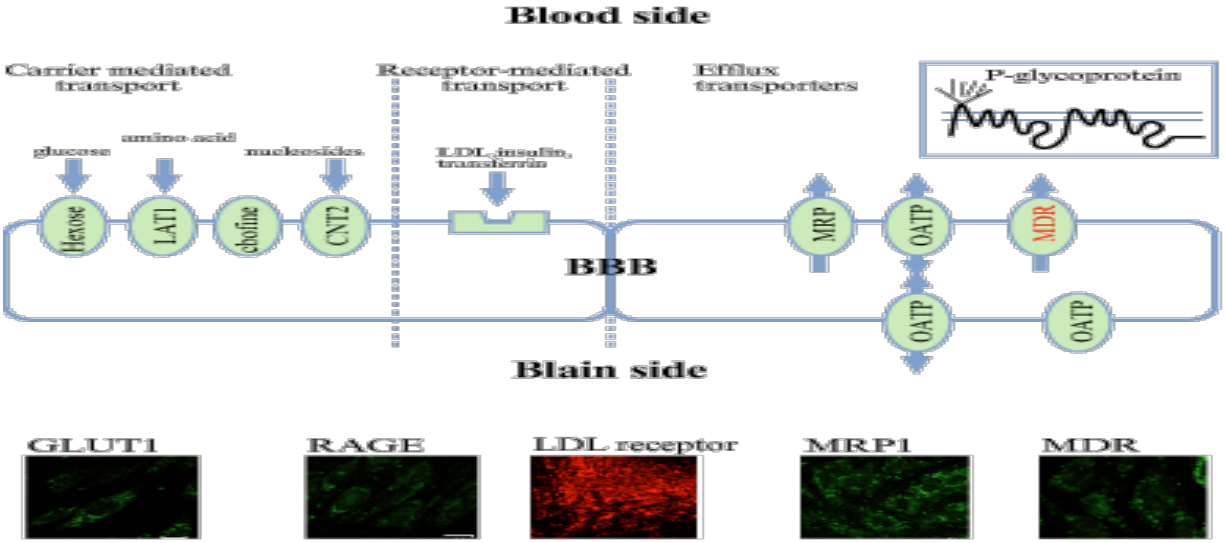

Microvessels

- Transport and permeability studies from ions to macromolecules: effect of physiological or pathogenetic factors
- Paracellular barrier and cell polarity studies: TJ protein expression, distribution, polarized distribution of transport proteins, receptors, enzymes etc
- Studies on endo- and transcytosis, receptor-ligand interactions

- Drug transport, drug effect on permeability, localization of receptors, polarity of drug responses
- Co-culture studies: cell-cell and cell-matrix interactions
- Microbial pathogenesis: virus, bacteria, parasite attachment, invasion and penetration
- Compounds screening neuroimmune targets




## Expressions of BBB transporters in our 3D Human BBB Model



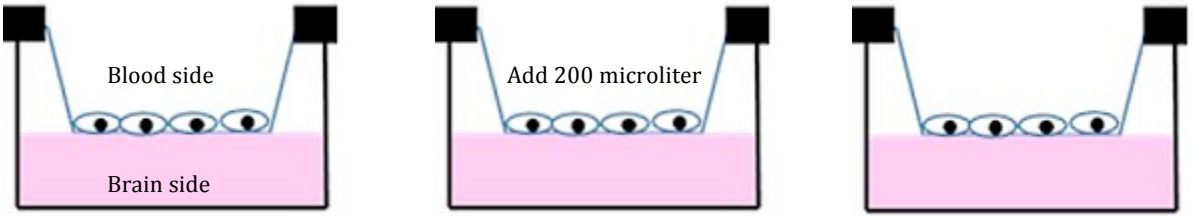

## Trans Endothelial Electrical Resistance (TEER) of our 3D Human BBB model

TEER in3D Human BBB model reaches more than 400 Ω x cm2, and maintains a plateau up to 6 days. TEER is measured at Day 3 after changing medium.

# Using Our 3D BBB model if frozen:

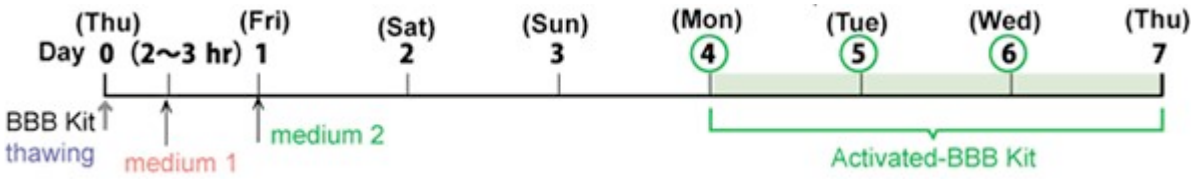
We deliver the 3D Human BBB model in frozen packaged with dry ice. The3D Human BBB model can be frozen and stored at -80°C. 4 days prior to your experiment, you just thaw your 3D Human BBB model stored at –80°C.

**Thawing the 3D Model:**




Add 1000 microliter

1. Day 0 thaw the model
2. Once thaw, remove old medium on both sides.
3. Add 200 microliters of new medium 37° C medium to the Blood side 4. Add 1000 microliters of new medium 37° C medium to the Brain side
5. Incubate for 2-3 hours in a 5% CO2 incubator.
6. After 2-3 hours, remove the media and repeat step 3 and 4.
7. Incubate overnight in a 5% CO2 incubator.
8. You can perform your experiment in days, 4, 5, 6, and 7 after thawing



## Storing of BBB model thawing-sol and incubation medium

BBB is stored at **-80** ℃, and can be used within one month. Thawing-sol and incubation medium are stored at below -**20**℃**.**

## 3. Protocol (procedure of activating BBB model)

thawing*[1~8] →medium 1*[9~10] →incubation*[11→] medium 2*[12~14] →incubation*[15] →experiment*[16] Medium 3

medium 1 Cat.# BBB-GM001; Blood Brain Barrier Growth Media 100mL

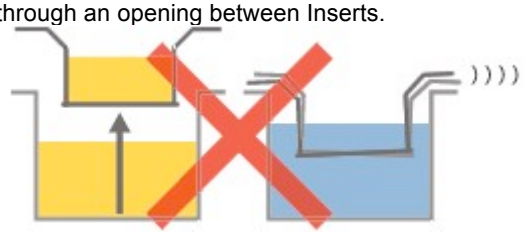
medium 2 Cat.#NMBBB001; Endo-Neuro-Pharmaceuticals Media 100mL

Medium 3 Cat.#TMBBB001; Blood Brain Barrier Transportation Media 100mL

### [ On thawing (day 0) ]

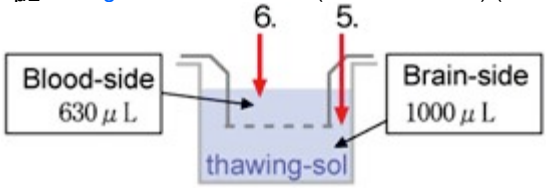
1. Warm thawing-sol to 37 ℃, prior to De-freezing and warm up BBB model.
   (Move frozen thawing-sol to 37 ℃ water-bath.)
2. Move thawing-sol to clean-bench.
3. Move a BBB model in frozen to clean-bench. Take off seals. (Do not take a minute.)
4. Wipe up water drops (humidity) on BBB model with clean papers. 5. Add 1,000 µL thawing-sol to Brain-side (to all 12-wells)

through an opening between Inserts.



Do not touch membrane of insert with pipette, and do not move insert, during procedures of #1 to #5.

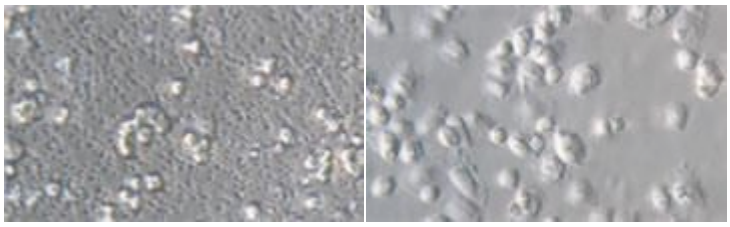
6. Add 630 µL thawing-sol to Blood-side (inside of Insert) (to all 6,12 or wells).




7. Stir up gently Blood-side (inside of Insert) with a pipette, 5 to 10 times.
   Wipe up humidity on surface and bottom of BBB model.
   (Do not stir up Brain-side.)
8. Incubate BBB model for 2 to 3 hrs. in $CO_2$ incubator. During this incubation, warm medium 1 to 37 ℃
9. See cells with inverted microscope.

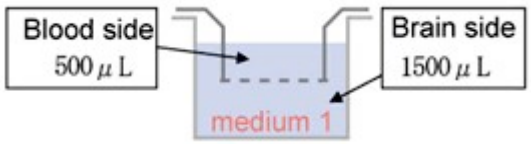
   Endothelial cells on Polycarbonate membrane of BBB model(PC-12) cannot be seen by microscope, therefore microscopic examination for astrocytes on bottom side of lower compartment has to be done to check cellproliferation.

   BBB model



endothelial cells (high magnification) astrocytes (high magnification)

10. Remove thawing-sol from Brain-side, and add 1,500 µL medium 1 (red arrow), then remove thawing-sol from Blood-side, and add 500 µL medium 1.(Do not touch cells, carelessly. Add medium 1, very gently.)

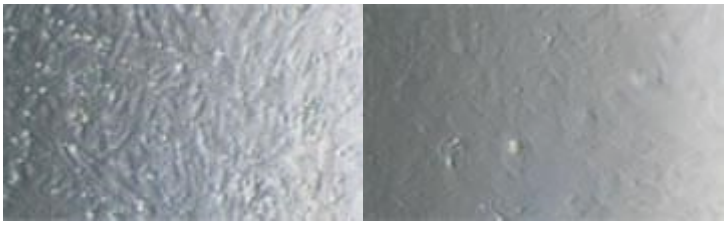



11. Incubate BBB mode with medium 1 $CO_2$ incubator, overnight.

**[ On day 1 (the next day after thawing of BBB model) ]**

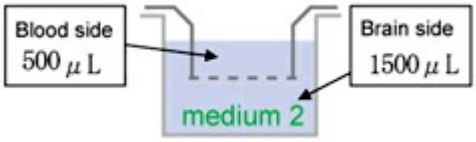
12. See astrocytes through polycarbonate membranes with inverted microscope.

BBB model



endothelial cells (low magnification) astrocytes (low magnification)

13. Warm medium 2 to 37 ℃ in water-bath (Move frozen medium 2 to 37 ℃ water-bath. )
14. Remove medium 1 from Brain-side, and add 1,500 μL medium 2 (red arrow), then remove medium 1from Blood-side, and add 500 μL medium 2 (Do not touch cells, carelessly. Add medium 2 very gently.)



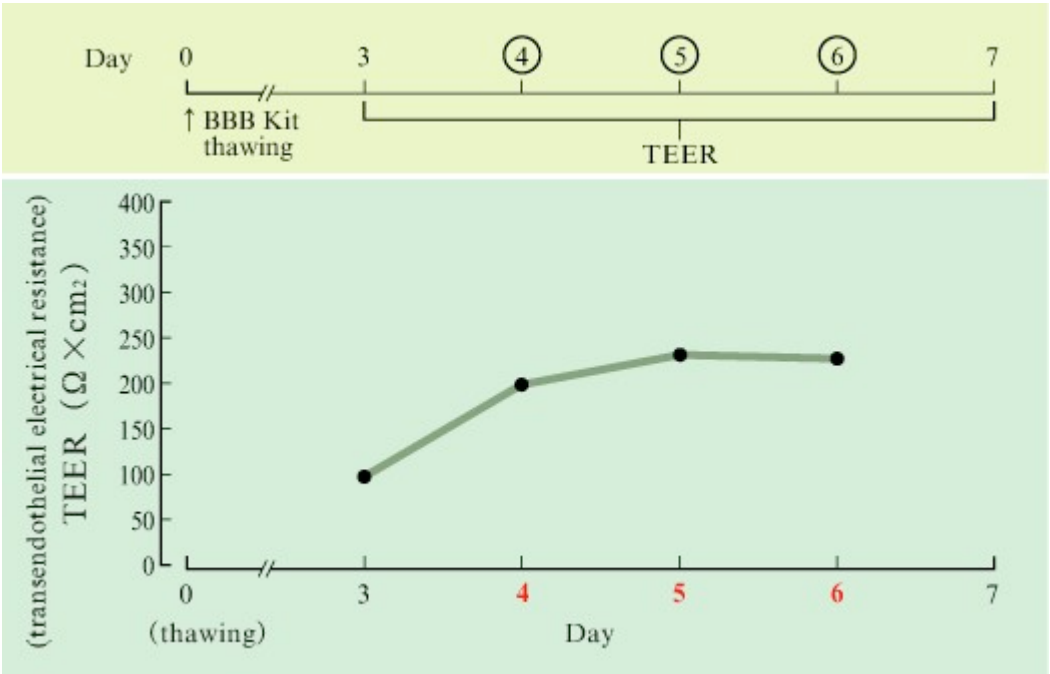

15. Incubate BBB model with medium 2 in $CO_2$ incubator for 3 days. (from thawing day (Day 0) to Day 4)
16. On Day 4, BBB model is activated functionally, and maintains BBB function until Day 7. Use activated-BBB model on Day 4. (You can store activated- BBB model in $CO_2$ incubator at 37 ℃. We recommend you use the BBB model until Day 6.)

17. Use Medium 3 Cat.#TMBBB001; Blood Brain Barrier Transportation Media 100mL when testing the penetration of a molecule through the brain endothelial and pericytes layer of BBB.

## TEER (trans endothelial electrical resistance) in BBB model

TEER in BBB model reaches more than 150 Ω×cm$^2$, and maintains a plateau up to 7 days.
(Thawing-sol and medium 1, 2 do not contain cAMP and its analogs.) *BBB model can be used from Day 4 to Day 6 after thawing.

**BBB model**




# 3D Human Blood Brain Barrier Permeability assay

When testing the penetration of a molecule through the brain endothelial and pericytes layer of BBB representing the BBB, in a blood-to-brain direction, the molecule is applied to the upper (luminal, blood-side) compartment of the insert. Transport is

measured after a given time (⊿T) by detecting the amount of compound from the lower (basal, brain-side) compartment.


⊿t

**Test compounds (not provided)**

- **Stop watch (not provided)**
- **Orbital Shaker (100 rpm) in Incubator 37℃ (not provided)**

# Summary:

Check TEER of activated BBB Make sure TEER >150Ω x cm²

→ Prepare Assay buffer, test compounds, wash plate and assay plate → Permeability Assay

→ Measure concentration of test compound in lower compartment → Calculate peameability coefficient

TEER measurement :

TEER (Ω x cm²) = (Total R - Blank R) x 0.33.

Blank insert should be soaked with medium (DMEM) prior to its use for accurate reading Only use inserts with TEER value of 150 Ω x cm² or more for assay.

Assay :

- Make sure final concentration of DMSO is equal to or less than 0.2% (v/v) when test compound is dissolved in DMSO. Concentration of test compound used should not be at the concentration which cause any cytotoxic effects. For unknown compounds start with 1μM and adjust concentration as required.
- Use orbital shaker (100 rpm) during incubation period for obtaining accurate result.

## Check TEER of activated 3D BBB Model

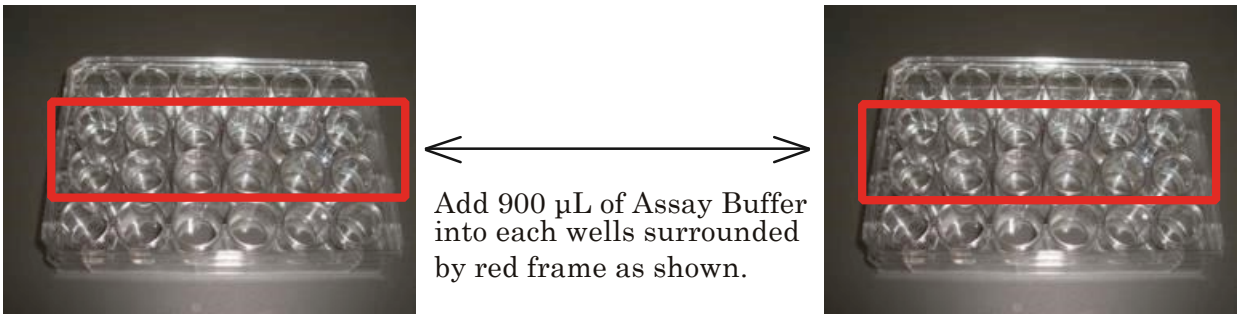
Measure TEER of activated BBB. Make sure TEER > 150 Ωx $cm^2$ before assay. Please refer to the protocol for TEER measurement of BBB.

## Prepare Assay Buffer (DPBS-H), test compounds, wash & assay plate

1. Preparation of Assay Buffer (DPBS-H); Mix as follows.

| | |
|---|---|
| 10 x Dulbecco's PBS (Ca+/Mg+) | 10 mL |
| 1M HEPES (pH 7.0 - 7.6) | 1 mL |
| D-glucose | 0.45 g |
| distilled water | 89 mL |
| total | 100 mL |

2. Prepare test compounds in Assay Buffer to appropriate concentration, then keep them at 37 °C.
3. Add 900 µL of Assay Buffer into 12 wells of wash and assay plate, then keep them at 37 °C.




Wash Plate

Assay Plate

## Permeability Assay

1. Remove all 12 inserts from BBB into wells of the Wash Plate containing assay buffer with clean tweezer.

activated BBB Model

Remove inserts into the Wash Plate.

Wash Plate

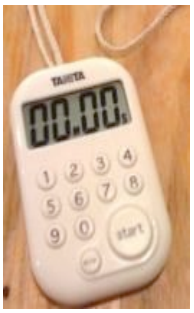
2. One insert at a time:

- Pick it up with tweezer and remove culture media from luminal side, then return the insert into Wash Plate.
- Add 200 µL of Assay Buffer containing test compound which is kept at 37 °C.
  Note: Do not wash inside of the insert with Assay Buffer.
- Quickly transfer the insert from Wash Plate into Assay Plate.
- As you transfer the first insert into the Assay Plate start stopwatch.
- Place on a shaker inside an incubator when all 12 inserts are transferred to Assay Plate.
- Incubate at 37 °C, 100 rpm, for <30 minutes.


Plate with Assay with Stop Watch

Wash insert Plate inserts

containing Test Compound



3. Collect Assay Buffer from inserts and Assay Plate for measuring concentration of Test Compound (Apical and Basal concentration). Make sure you perform pipetting action x10 times to have no test compounds remaining at the bottom of the well.

Mix collected sample with Vortex. Measure concentration of Test Compound and determine permeability coefficient using Excel form provided.

1. We recommend to use Millipore Plate (Millipore corporation #PIMW S24 50).

2. The volume of Assay Buffer and Test Compound Dissolved-Assay Buffer varies if other Assay Plate is used. Please refer below.

3. Assay Time:

The amount of Test Compound which penetrate through to the brain-side will be greater when assay time is increased. Although concentration measurement will be easier this way especially when detection-limit is low, the barrier-function (tight junction-function) of BBB Kit will deteriorate with time and hence increase paracellular transport of Test Compound. For this we recommend completing the assay within 30 minutes for accurate evaluation.

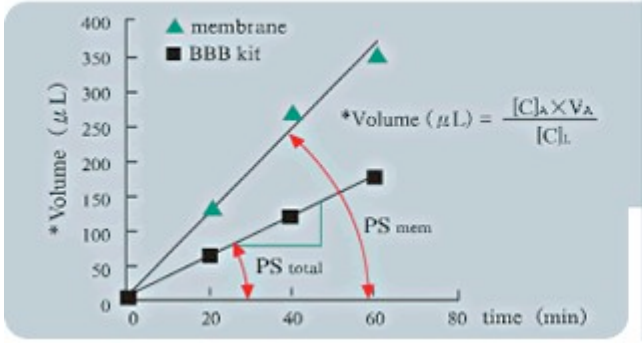
| | Blood-side Volume (µL) | Brain-side Volume (µL) |
|---|---|---|
| **Millipore Plates** | 100 | 600 |
| | **200*** | **900*** |

| | 300** | 1,200** |
|---|---|---|
| Corning Plates | 100 | 1,000 |
| | 200 | 1,300 |
| | 300 | 1,600 |
| BD Plates | 100 | 1,000 |
| | 200 | 1,300 |
| | 300 | 1,600 |

*Recommendation to use for permeability assay. **Volume used for BBB model activation.

# Analysis:

1. **Calculation of apparant permeability**



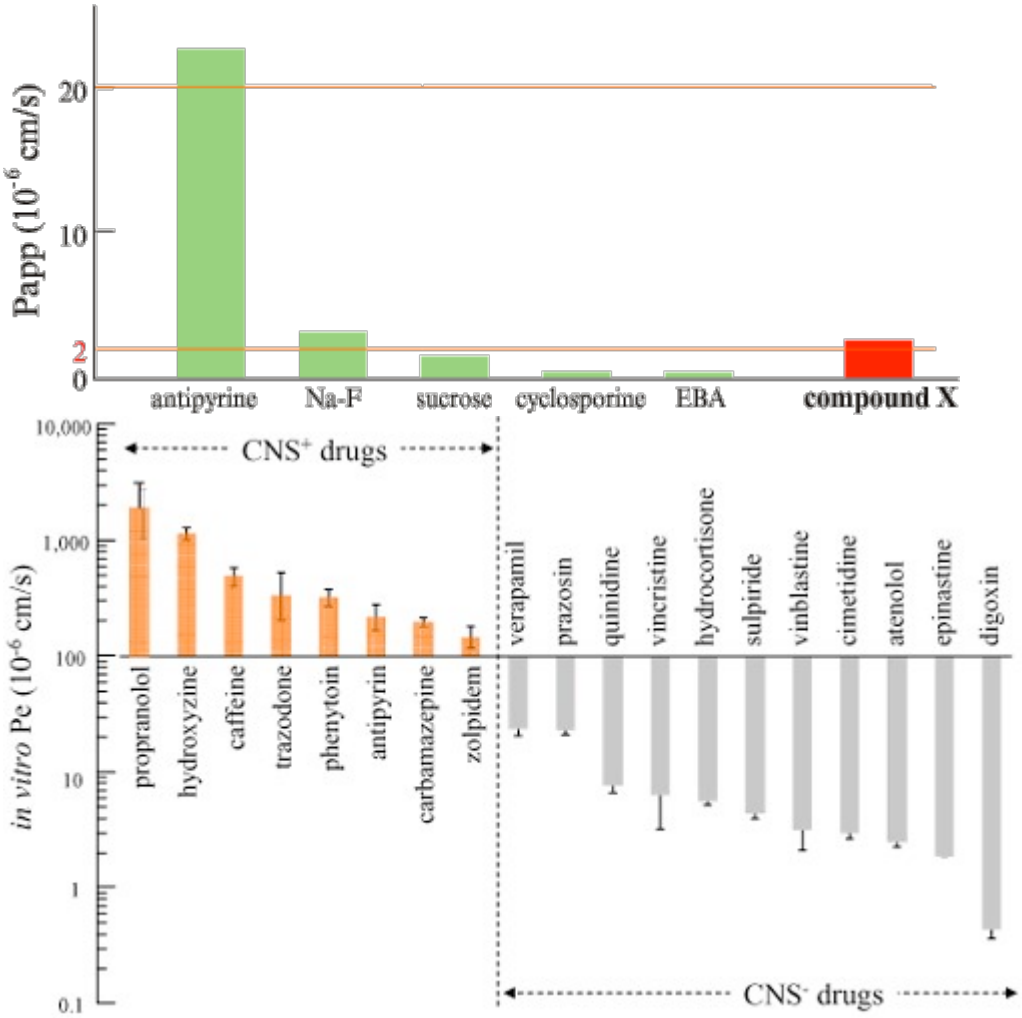

**Caluculation of apparent permeability**

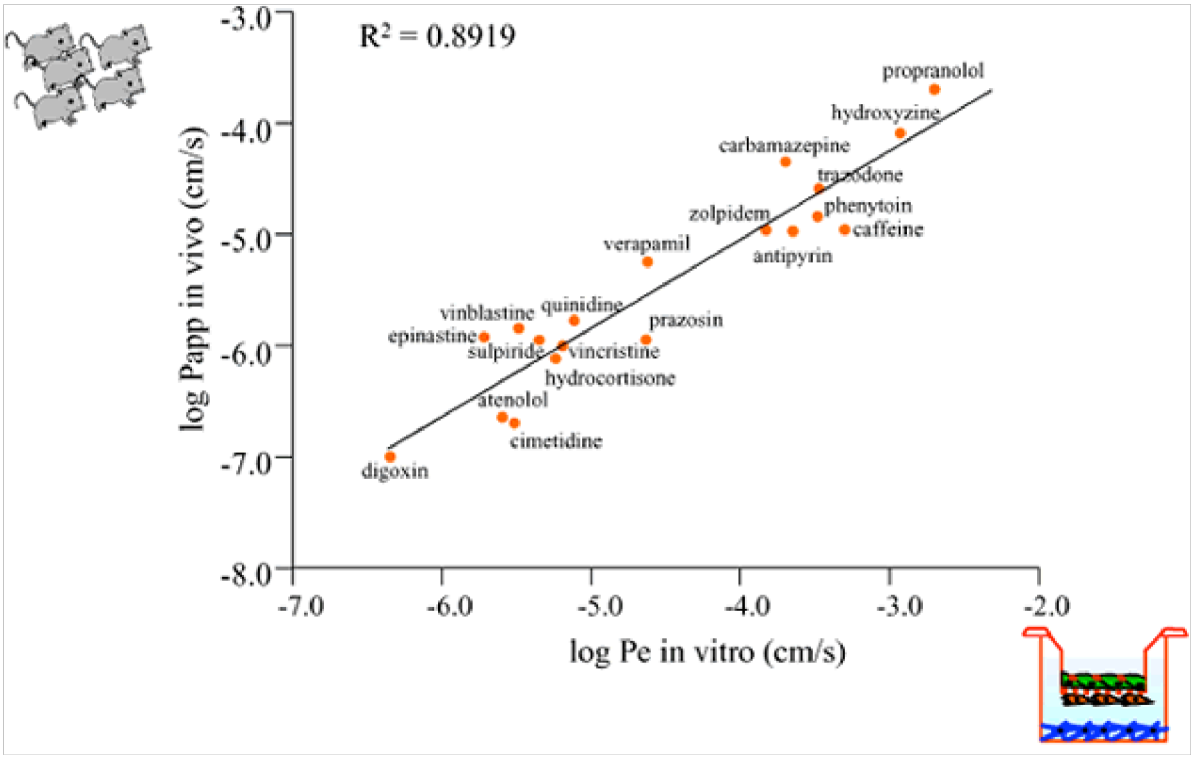
$$\text{①}\quad \frac{1}{PS_e} = \frac{1}{PS_{total}} - \frac{1}{PS_{mem}}$$

$$P_e\,(cm/min) = \frac{PS_e\,(\mu L/min)}{A\,(cm^2)}$$

$$\text{②}\quad P_{app}(cm/s) = \frac{V_A}{A \times [C]luminal} \times \frac{\Delta [C]Abluminal}{\Delta T}$$

2. **Evaluation of Papp**






We obtained a very good correlation between the BBB and in vivo permeabilities of drugs. You can evaluate the BBB-permeability by our BBB model, quantitatively. When you design molecular modifications of your compound or vectors carrying your compound into the brain, you can easily evaluate the BBB-permeability, quantitatively.

| | Papp（×$10^{-6}$cm/s） | Permeability | ex) |
|---|---|---|---|
| Permeabilitis | >20 | very good | Antipyrine |
| | 10～20 | good | - |
| | 2～10 | low | Na-F |
| | <2 | very low | ESA, sucrose |

*Transwell® is trademark of Corning, Incorporated, Corning, NY, USA.

# Plate and Wells information:

| | | |
|---|---|---|
| Membrane material | Polyethylene Terephthalate | Polyester |
| Pore Size (µm) | 3.0 | 0.4 |
| Membrane Diameter (mm) | 6.5 | 12 |
| Membrane Surface Area ($cm^2$) | 0.33 | 1.12 |
| Apical Volume (µL) | 200/300 | 500 |
| Basolateral Volume (µL) | 900/1200 | 1500 |
| Height of insert (mm) | 16 | - |
| Pore Density (pores/$cm^2$) | $2 \times 10^6$ | $4 \times 10^6$ |
| Membrane Thickness (µm) | 9 | 10 |
| Optical Property | Translucent | Clear |
| Cell visibility | Poor | good |

## 3D Human BBB Model Comes with:

- 2X50 ml culture Medium if frozen
- 1X50ml culture Medium if not frozen
- Room Temp or Frozen plate with insert