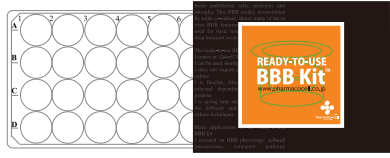
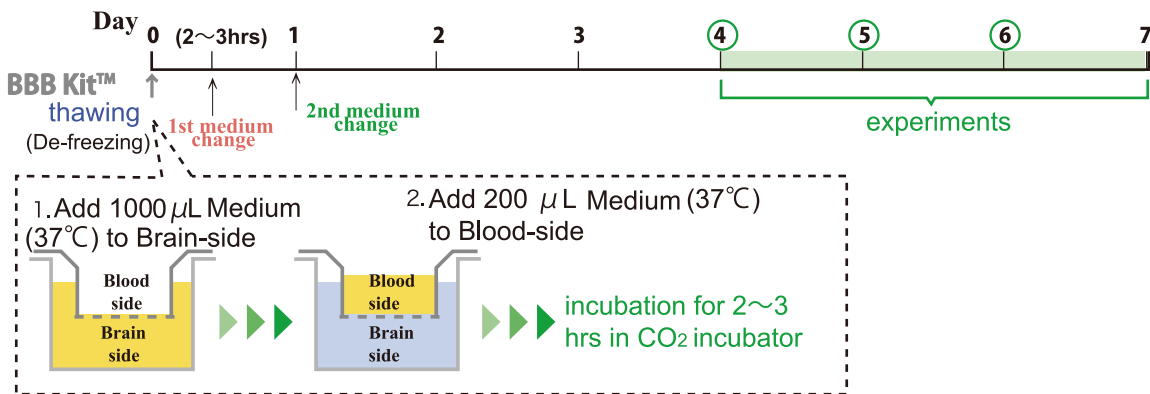




- Package
- ⊙ BBB Kit™ (MBT-24)
 - ⊙ Medium
 - ⊙ Blank insert (Millicell®)
 - ⊙ Protocol
 - ⊙ Quality Assurance
- 
- *Please check the contents.

Protocol: Thawing and Activation of BBB Kit™ (MBT-24)

1. Schedule after thawing BBB Kit™



Amounts of Medium (to be added)

	Thawing (De-freezing)	1st medium change	2nd medium change
Blood-side	200 µL	300 µL	300 µL
Brain-side	1000 µL	1200 µL	1200 µL

※ Medium Composition

10%PDS / DMEM F-12:

DMEM / Nutrient Mixture F-12 Ham (DMEM F-12) supplemented with

Fetal Bovine Plasma Derived Serum (PDS)	10% (v/v)
Heparin	1850 U/mL
basic Fibroblast growth factor (bFGF)	1.5 ng/mL
Insulin-Transferrin-sodium Selenite (ITS)	insulin 5 µg/mL transferrin 5 µg/mL sodium selenite 5 ng/mL
Hydrocortisone	500 nM
Gentamicin	50 µg/mL

2. Storing of BBB Kit™

BBB Kit™ (MBT-24) is stored at **-80°C**, and can be used within one month.
Medium is stored at below **-20°C**.

3. Procedure: Thawing and activating of BBB Kit™ (p2)

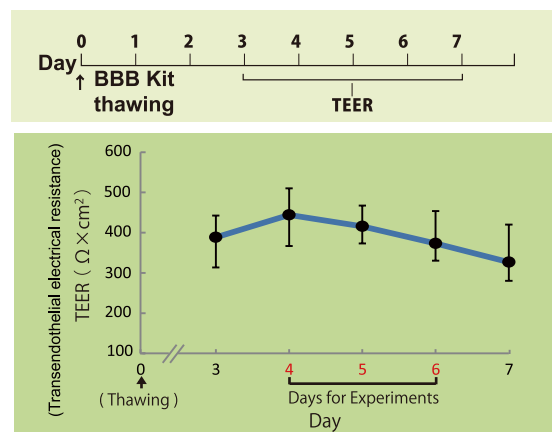
thawing^{*1~8} ⇒ 1st medium change^{*9~10} ⇒ incubation^{*11} ⇒ 2nd medium change^{*12~14} ⇒ incubation^{*15} ⇒ experiment^{*16}
(* protocol # see p2)

4. TEER (trans endothelial electrical resistance) in BBB Kit™

TEER in BBB Kit™ (MBT-24) reached more than 150 Ω×cm², and maintained a plateau up to 7 days from thawing.

* Medium does not contain cAMP and its analogs.

* BBB Kit™ can be used from Day 4 to Day 6 after thawing.



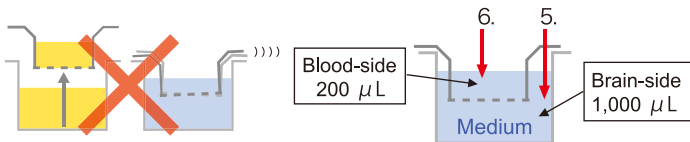
※ BBB Kit™ is not available for clinical diagnosis, examinations, and treatments.

3. Procedures (from thawing to activation of BBB Kit™)

[On thawing (day 0)]

1. Warm frozen Medium to 37 °C in water-bath, prior to De-freezing a BBB Kit™.
2. Move Medium from water-bath to clean bench.
3. Move a BBB Kit™ in a frozen state to clean-bench quickly. And then take off the seal.
4. Wipe up the waterdrops (humidity) on a BBB Kit™ with clean papers.
5. Add 1,000 μL Medium to Brain-side (to all 12-wells) through a space between insert and well.

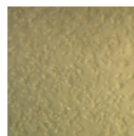
During steps from #1 to #6 on this manual,
 •Do not touch membrane of insert with pipette tip.
 •Do not move inserts.
 Otherwise, TEER in each well may not achieve more than 150Ω×cm².



6. Add 200 μL Medium to Blood-side (inside of the insert) (to all 12-wells).
7. Wipe up humidity on the surface and bottom of the BBB Kit™ with clean papers.
8. Incubate the BBB Kit™ for 2 to 3 hours in CO2 incubator. Warm Medium to 37°C during this step.
9. Look at astrocytes through a inverted microscope to confirm the attachment of cells to the bottom of well.



astrocytes

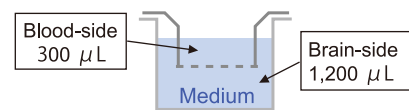
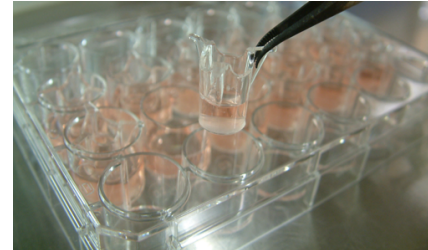


endothelial cells

Endothelial cells on Polyethylene terephthalate membrane of the insert can not be seen by microscope, therefore microscopic examination for astrocytes on the bottom side of lower compartment has to be done to check cell-proliferation.

10. ① Lift-up the insert using a pincet, and remove the present Medium of the brain-side.
 ② Remove the present Medium of blood-side, then return the insert to plate.
 ③ Add 300 μL new Medium to the blood-side very gently, and then add 1,200 μL new Medium to the brain-side.
 ④ Repeat ①~③ in each well.

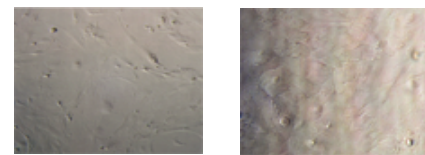
Notice : Do not touch cells or membrane of the insert.



11. Incubate the BBB Kit™ in CO2 incubator, overnight. Store remains of medium in the refrigerator.

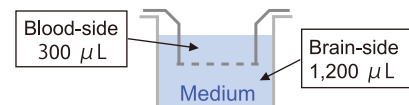
[On day1 (the next day after thawing of BBB Kit™)]

12. Look at astrocytes just below the insert through a inverted microscope.



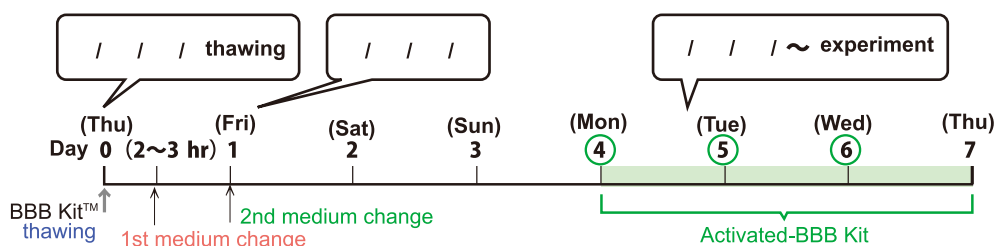
astrocytes

13. Warm Medium to 37 °C in water-bath.
14. Change medium in exactly the same way described at step 10.



15. Incubate the BBB Kit™ in CO2 incubator for 3 days *.
 * Day 0 (thawing day) to Day 4
16. On Day 4, BBB Kit™ is activated functionally, and maintains BBB functions until Day 7.
 We recommend you to use activated-BBB Kit™ during the period from Day 4 to Day6.

Schedule (example)



※ BBB Kit™ is not available for clinical diagnosis, examinations, and treatments.

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