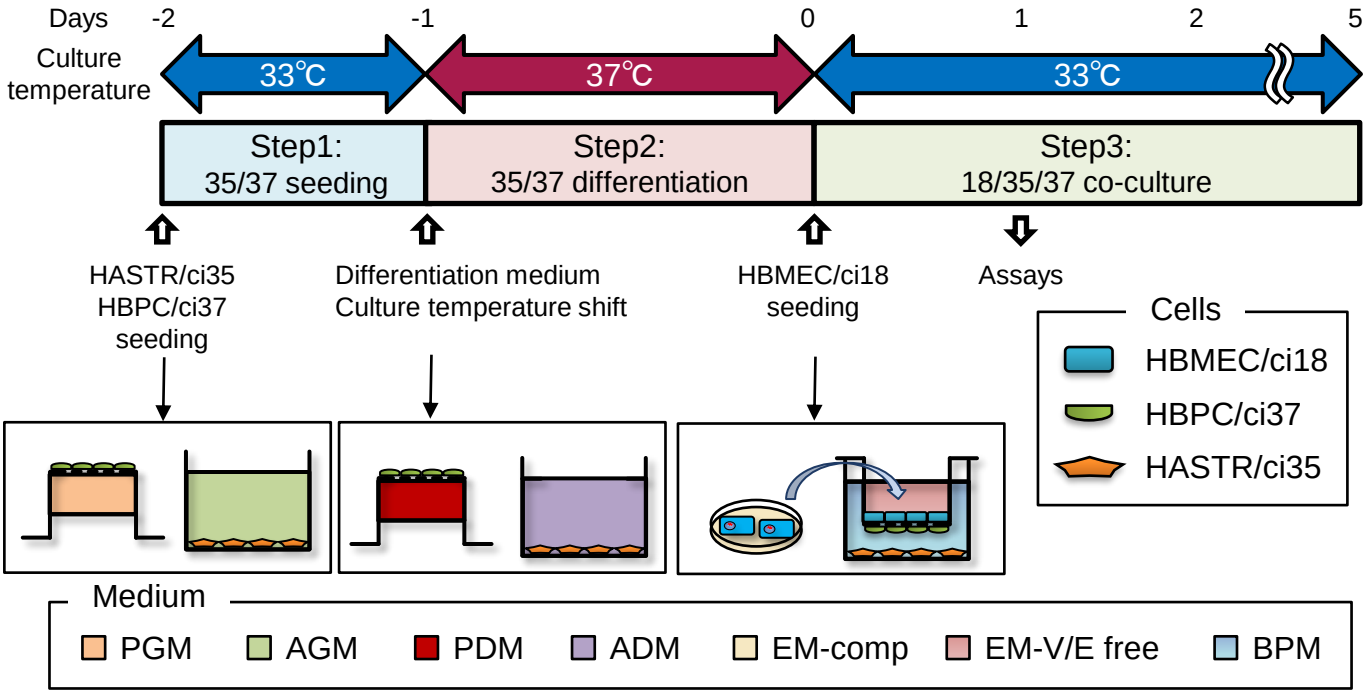
The hiBBB90 Model Setup Protocol

ver. 1.3

(updated on Mar. 25, 2021)

Overview & Medium composition



Medium name		PGM	PDM[*1]
Cells		HBPC/ci37	
Basal medium		Pericyte medium (Sciencell, #1201-b)	
Culture supplements			
Pen/st (#0503)	1%	+	+
FBS (#0010)	2%	+	–
Pericyte growth factor (#1252)	1%	+	+
Blasticidin S	4 μg/mL	+	–

[*1], Opitional. While we used FBS-containing medium in our initial work (Ito et al. 2019), you may change it to FBS-free medium.

Medium name		AGM	ADM[*2]
Cells		HASTR/ci35	
Basal medium		DMEM	
Culture supplements			
Pen/st	100 units/mL-100 μg/mL	+	+
N2 (apo)	1%	+	+
FBS	10%	+	–
Blasticidin S	4 μg/mL	+	–
dBcAMP	1 mM	–	+

[*2], it may be better to further add Glutamax or L-Alanyl-L-Glutamine as we did in our initial work (Ito et al. 2019).

Medium name		BPM
Cells		HASTR/ci35, HBPC/ci37
Basal medium		Neurobasal medium (Life Technologies #21103049)
Culture supplements		
Pen/st	100 units/mL-100 μg/mL	+
N2 (apo)	1%	+
L-glutamine	2 mM	+

Medium name		EM-comp	EM-V/E free
Cells		HBMEC/ci18	
Basal medium		VascuLife BM (#LEB-LM0002) / EBM-2 BM (#CC-3156)	
Culture supplements			
VascuLife VEGF Lfactors (#LEK-LS1020) or EGM-2 SingleQuots Supplements (#CC-4176) [*3]		+	+
hFGF-b	5 ng/mL	+	+
Ascorbic acid	50 μg/mL	+	+
HC	1 μg/mL	+	+
hIGF-1	15 ng/mL	+	+
hEGF	5 ng/mL	+	–
VEGF	5 ng/mL	+	–
Heparin	0.75 unit/mL	+	+
L-glutamine	10 mM	+	+
FBS	2%	+	+
Pen/st	100 units/mL-100 μg/mL	+	+
Blasticidin S	4 μg/mL	+	–

EM-comp, Endothelial complete medium;
EM-V/E free, VEGF- and hEGF-free EM-comp
FBS, fetal bovine serum
HC, hydrocortisone
hFGF-b, human fibroblast growth factor-basic
hIGF-1, human insulin-like growth factor-1
hEGF, human epidermal growth factor
VEGF, vascular endothelial growth factor
Pen/st, penicillin streptomycin
AGM, astrocyte growth medium
ADM, astrocyte differentiation medium
DMEM, Dulbecco's modified Eagle medium
dBcAMP, dibutyryl cyclic adenosine monophosphate
PGM, pericyte growth medium
PDM, pericyte differentiation medium
BPM, brain parenchyma medium

[*3], other types of endothelial medium may be used.

Summary of each preparation step (for 12-well format)

Day

-2

33℃

-1

37℃

0

33℃

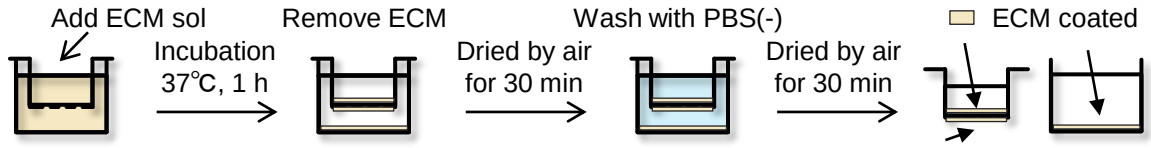
1

Step1: 35/37 seeding

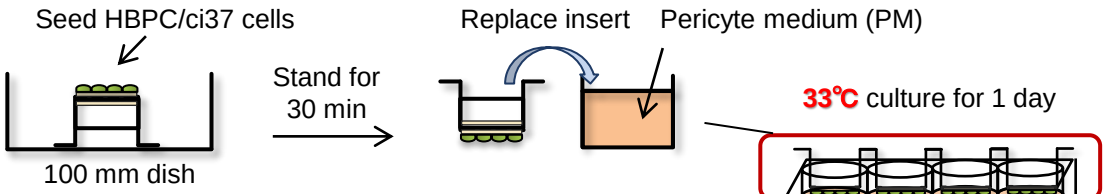
Step2: 35/37 differentiation

Step3: 18/35/37 co-culture

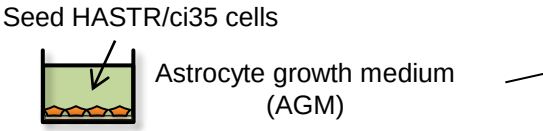
Step1-1: Extracellular matrix (ECM) coating



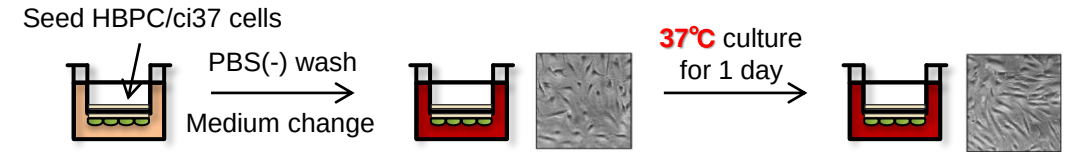
Step1-2: HBPC/ci37 (pericyte) seeding



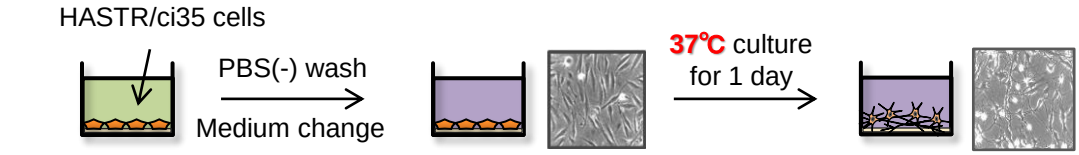
Step1-3: HASTR/ci35 (astrocyte) seeding



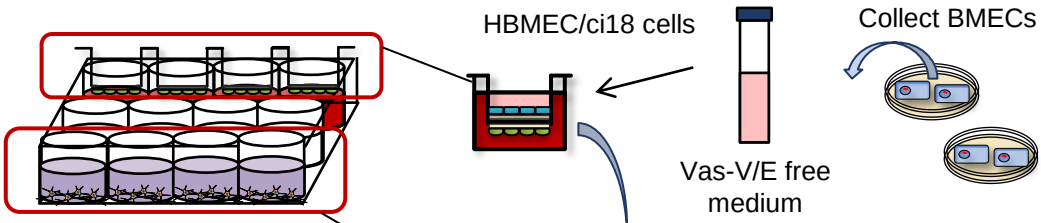
Step2-1:HBPC/ci37 (pericyte) differentiation



Step2-2: HASTR/ci35 (astrocyte) differentiation

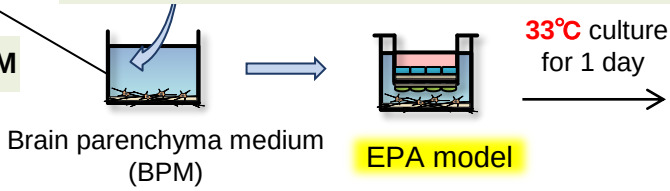


Step3-1:HBMEC/ci18 (endothelial cells) seeding



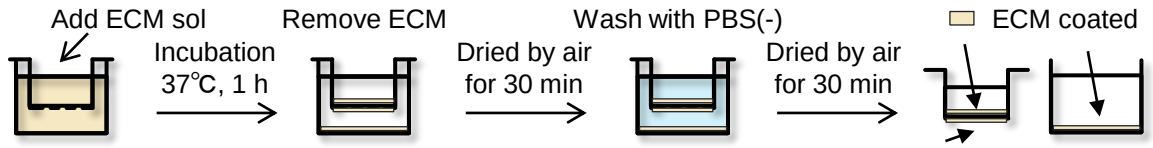
Step3-3:Combine insert and receiver plate

Step3-2:Medium change to BPM



Step1-1: Extracellular matrix coating

Step1-1: Extracellular matrix (ECM) coating



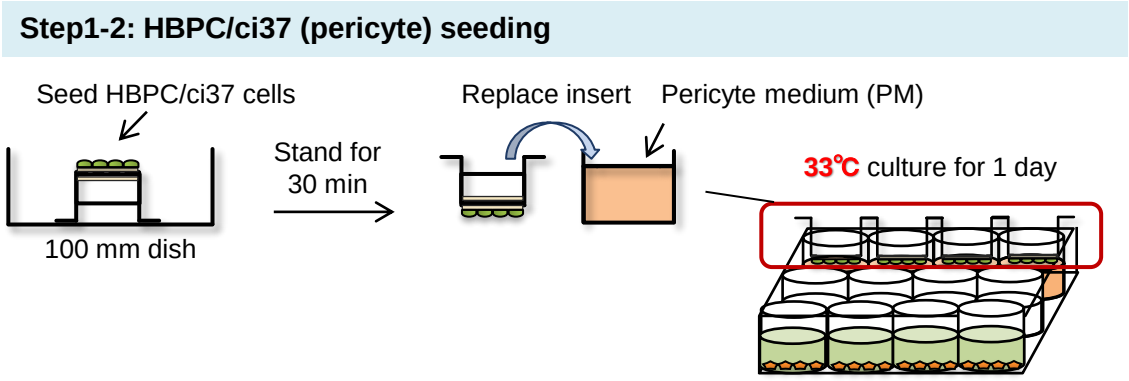
- Set the inserts on the culture plates※1, then add 250 µL and 750 µL ECM solution※2 to the apical and the basolateral side, respectively. Stand at 37℃ for one hr.
- Remove the ECM solution and dry the inserts and the plates by air for 30 min.
- Wash the inserts and the plates with PBS (-).
- Dry the inserts and the culture plates well for 30 min※3.

※1
In our study, 12-well culture plate and insert (translucent polyethylene terephthalate, 0.4 µm high-density pores, BD Falcon) are used. However, other culture materials can be used.
For 24-well, adjust the volume proportionally.

※2
ECM solution is a mixture of type-IV collagen and fibronectin (final concentration: 0.1 mg/mL of each).
Prepare at the time of use.

※3
Make it sure that the inserts are completely dried out.

Step1-2: HBPC/ci37 (pericyte) seeding



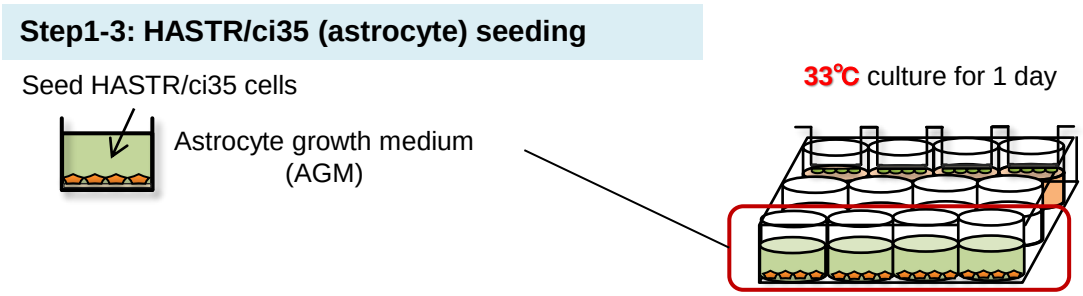
- Place the insert in a container (which is sufficiently larger than the insert: e.g. 100-mm dish) in an upside-down manner.
- Prepare 300 µL HBPC/ci37 cell suspension at 3.0×10^4 cells/cm^2 using the pericyte medium and place them **gently** on the insert※4.
- Stand it in the clean bench until the cells adhere (approx. 30 min)※5.
- Turn over the insert upside down and **carefully** set it in the culture plate, where the pericyte medium are filled in advance※6.
- Culture at **33℃** in 5% CO_2/95% air.

※4
Seed gently, or the cell suspension may fall into the bottom. For a 24-well format, the volume should be adjusted according to the manufacturer's instruction.

※5
No problem if a small portion of cell suspension drops to the bottom during the incubation, unless most cells remain attached.

※6
Confirm that a large number of the cells cannot be found in the bottom of the plate with a microscope.

Step1-3: HASTR/ci35 (astrocyte) seeding

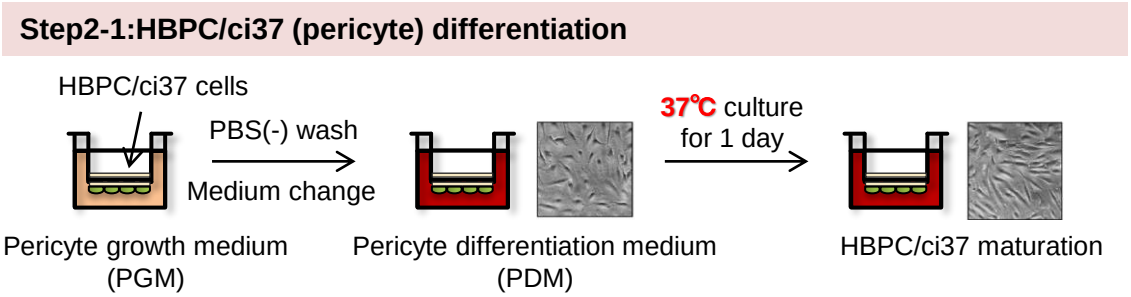


- Prepare 750 µL HASTR/ci35 cell suspension at 2.5×10^4 cells/cm^2 using the astrocyte growth medium and place it on the culture plate※7.

- Culture at **33℃** in 5% CO2/95% air.

※7
Important! To prevent the cells from clustering in the center of the plate, shake gently the plate and make them uniformly attached. For a 24-well format, the volume should be adjusted according to the manufacturer's instruction.

Step2-1: HBPC/ci37 (pericyte) differentiation



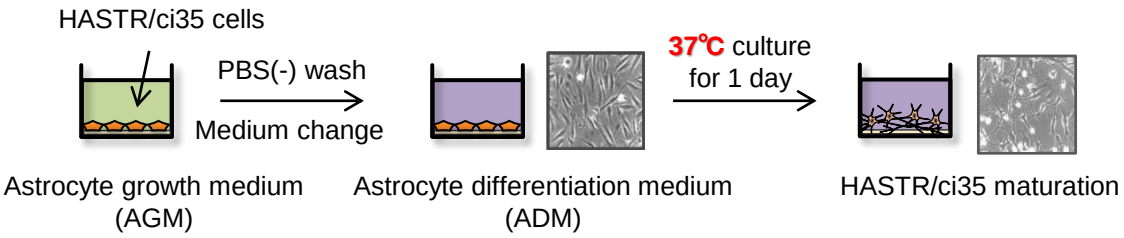
- Confirm that a large number of HBPC/ci37 cells are not found in the culture plate with a microscope.
- Gently wash twice with PBS (-).
- Change the medium from PGM (for cell proliferation) to PDM (for cell differentiation)※8.
- Culture at **37℃** in 5% CO2/95% air※9.

※8
Please note that in our published paper, we use PGM only. While we assume PDM would be better, you can follow the published protocol. In such case, please skip the medium change and just do temperature change alone.

※9
Make sure that the temperature is set at 37℃.

Step2-2: HASTR/ci35 (astrocyte) differentiation

Step2-2: HASTR/ci35 (astrocyte) differentiation



- Gently wash twice with PBS (-)※10.
- Change the medium from AGM (for cell proliferation) to ADM (for cell differentiation).
- Culture at **37℃** in 5% CO_2/95% air※11.
- Confirm that the HASTR/ci35 cells show differentiated morphology at several hours after the medium change※12.

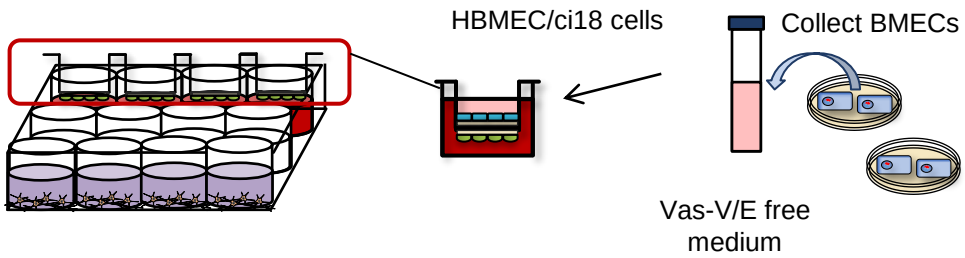
※10
Important! Wash out FBS completely, or the differentiation would be insufficient.

※11
Make sure that the temperature is set at 37℃.

※12
Please refer to Kitamura et al. J Pharmacol Sci. 2018;137(4):350-358.

Step3-1: HBMEC/ci18 (endothelial cells) seeding

Step3-1:HBMEC/ci18 (endothelial cells) seeding

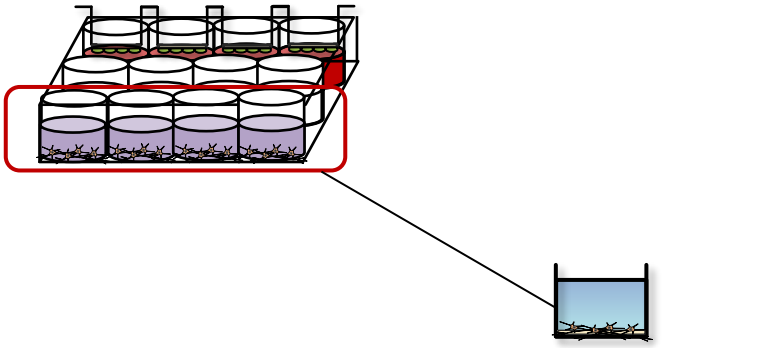


- Prepare 750 µL HBMEC/ci18 cell suspension at 1.3×10^5 cells/cm^2 using the VEGF/EGF-free endothelial medium, and gently place it on the insert※13.

※13
For a 24-well format, the volume should be adjusted according to the manufacturer's instruction.

Step3-2: Medium change to BPM

Step3-2:Medium change to BPM



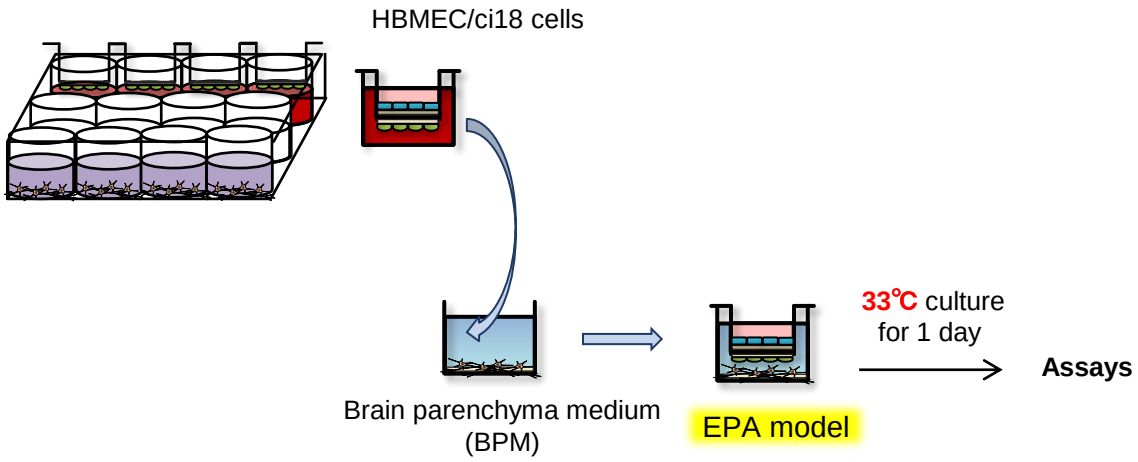
Brain parenchyma medium
(BPM)

- Change the medium from ADM to BPM (2250 µL) ※14.

※14
For a 24-well format, the volume should be adjusted according to the manufacturer's instruction.

Step3-3: Tri-coculture

Step3-3:Combine insert and receiver plate



- Carefully transfer the inserts to the culture plate and start co-culturing.
- Culture at **33℃** in 5% CO_2/95% air※14.

※14
Make sure that the temperature is set at 33℃.

The hiBBB90 model setup protocol ver. 1.3 Mar. 25, 2021.

This is the first version and will be updated without any prior notices. Any questions and comments would be greatly appreciated.

Contact Information:

Tomomi Furihata, Ph.D.
Department of Clinical Pharmacy and Experimental Therapeutics
School of Pharmacy
Tokyo University of Pharmacy and Life Sciences
1432-1 Horinouchi, Hachioji, Tokyo, JAPAN 192-0355
Tel/Fax: +81-42-676-8969/5809
tomomif@toyaku.ac.jp