

Human Blood Cell (hBLD) Reprogramming Kit

For induction of human iPSCs from Peripheral Blood Mononuclear Cells (PBMCs)

Catalog Number: ST01005

Size: 5 reactions

Human blood cell is one of the ideal sources for iPSC cells reprogramming in both basic research and clinical application. Peripheral Blood Mononuclear Cells (PBMCs) are blood cells with a round shaped nucleus, such as monocytes and lymphocytes, with the lymphocyte population consisting of T cells, B cells and NK cells which can be isolated from whole blood through density gradient centrifugation using Ficoll. Reprogrammed iPSC cells from normal or diseased human PBMCs can be used for exploring the mechanism of disease, drug screening and discovery, toxicity test for new drug, as well as cell therapy in future. Optimized conditions for iPSC cell reprogramming are crucial and beneficial for iPSC cell studies and its application.

Product Description

Stemmera™ Human Blood Cell (hBLD) Reprogramming Kit is a ready-to-use and fully chemically defined reprogramming kit which composed of necessary reagents and tools intended for culturing human PBMCs and induction of pluripotent stem cells (hiPSCs) from PBMCs, as well as maintenance of iPSCs under completely serum-free and feeder-free conditions.

Product Component

Component	Size	Storage	Catalog #
Human Blood Cell Reprogramming Kit-I			
Non-Enzymatic Cell Dissociation Solution	100 ml	2°C to 8°C	ST01005-S1
Blood Cell Reprogramming Basal Medium	2 x 500 ml	2°C to 8°C	ST01005-S2/BM
DMEM/F12	60 ml	2°C to 8°C	ST01005-S3
Human Blood Cell Reprogramming Kit-II			
Blood Cell Basal Medium	2 x 25ml	-20°C	ST01005-S4/BM
Blood Cell Supplement	61 µl for 25ml of BM	-20°C	ST01005-S4/S
Polybreen	10 µl	-20°C	ST01005-S5
Matrix	5 x 100 µl	-20°C	ST01005-S6

Blood Cell Reprogramming Supplement	2 x 3.5 ml	-20°C	ST01005-S2/S
Rock Inhibitor (1000x)	500 µl	-20°C	ST01005-S7
Blood Cell Cryopreservation Solution	10 ml	-20°C	ST01005-S8
Serum-Free Cryopreservation Solution for hPSC	20 ml	-20°C	ST01005-S9
Human Blood Cell Reprogramming Kit-III			
Cell Culture Plates	1 x 24 well	RT	ST01005-S10-24
Cell Culture Plates	1 x 12 well	RT	ST01005-S10-12
Cell Culture Plates	4 x 6 well	RT	ST01005-S10-6
Cell Counter Slides	3 pcs	RT	ST01005-S11
Trypan Blue	300 µl	RT	ST01005-S12
Reprogramming Factors	/	/	Not supplied

Note: Sendai Virus reprogramming factors from Life Technologies Inc is strongly recommended, Cat # A16517

Mixture of Blood Cell Reprogramming Basal Medium and its Supplement is a ready-to-use medium for reprogramming human PBMCs to iPSCs, as well as suitable for expansion and maintenance of human iPSCs after reprogramming under serum-free and feeder-free condition.

Mixture of Blood Cell Basal Medium and its Supplement is the ready-to-use medium for culturing PBMCs in suspension *in vitro*.

Matrix dilution: Dilute the matrix with cold plain DMEM/F12 medium (Stemmera, Cat # ST01005-S3) at ratio 1:500 on ice for coating the plates for culturing iPSCs under Serum-free/Feeder-free condition.

Storage and Handling

Stemmera™ Human Blood Cell (hBLD) Reprogramming Kit is shipped separately with blue ice pack, dry ice and room temperature. Store all components in appropriate



condition following manual instruction upon arrival and until the expiration dates stated on the product label. Avoid multiple freeze/thaw cycles. The complete medium should remain stable for 4-6 weeks at 2-8°C. Make aliquot of complete medium for daily use, avoid warm up cycles. Protect from light is preferred.

Product Use

This product is intended for *in vitro* use and research use only. Not intended for human or animal diagnostic or therapeutic uses.

General Precautions

1. Use aerosol barrier tips. Change tips after each use.
2. Always use fresh, clean gloves and wear lab coats.
3. Material Safety Data Sheet (MSDS) is available online.
4. Clean working space with 70% ethanol or other suitable disinfectant.

Culture Conditions

Media: Stemmera™ Human Blood Cell (hBLD) Reprogramming Medium, Stemmera™ Human Blood Cell Growth Medium

Cell: human induced Pluripotent Stem Cells (hiPSCs)

Culture type: Suspension culture, Single cell passaging and adherent culture

Temperature range: 37°C

Incubator atmosphere: Humidified atmosphere at 5% CO₂ and/or 5% O₂. Ensure proper gas exchange and minimize exposure

Protocol

• Preparation of complete Human Blood Cell Reprogramming Medium

Add 3.5 ml of frozen Blood Cell Reprogramming Supplement (Stemmera, Cat # ST01005-S2/S) in 500 ml of Blood Cell Basal Medium (Cat # ST01005-S2/BM), mix well and filter with 0.22µm filter.

• Preparation of complete Human Blood Cell Culture Medium

Add 61µl of Blood Cell Supplement (Stemmera, Cat # ST01005-S4/S) into 25 ml of Blood Cell Basal Medium (Stemmera, Cat # ST01005-S4/BM), mix well and filter with 0.22µm filter.

• Coat the vessels with Matrix (Cat# ST01005-S6)

For reprogramming of human PBMCs and maintenance of iPSCs, use Matrix (dilution ratio: 1:500) in the kit to coat the vessels and incubate in a 37°C, 5% CO₂ incubator for at least 30 minutes or

overnight. The coated vessels can be used in a week. Before use, remove all Matrix immediately and replace with pre-warmed complete medium with Rock Inhibitor (ST01005-S7, 1:1000 dilution).

- Aliquot and Pre-warm complete medium at room temperature for daily use.

Note: Complete Human Blood Cell (hBLD) Reprogramming Medium and complete Blood Cell Growth Medium should remain stable for 4-6 weeks when stored at 2 to 8°C in the dark within the expiration date of all components.

Culture Human PBMCs

1. Rapidly thaw one vial of 1-2x10⁶ of frozen human PBMCs in 37°C water bath.
2. Gently pipet the entire contents of the cryovial into a sterile 15 ml conical tube with Blood Cell Basal Medium (Cat # ST01005-S4/BM).
3. Centrifuge 15 ml conical tube at 1000 rpm for 5 minutes.
4. Aspirate and discard supernatant. Take extreme care not to disturb cell pellet.
5. Resuspend the cells in 1 ml of pre-warmed complete Blood Cell Growth Medium (Stemmera, Cat # ST01005-S4 with supplements).
6. Transfer the cells into 12-well plate and add sufficient complete medium to the wells (1 ml of medium per well of 12-well plate).
7. Incubate the cells in a 37°C, 5% CO₂ incubator overnight
8. Change 50% of medium the following day and keep culturing the cells by changing 50% of complete blood culture medium every other day for 6-8 days for reprogramming.

Reprogramming Human PBMCs

1. Once the cells cultured for 6-8 days, the cells are ready for reprogramming.
2. Day 0: On the day of reprogramming, transduce 2x10⁵ PBMCs in 24 well plate (non-Matrix coated) with reprogramming factors (Sendai Virus, Lentivirus, Retrovirus, Small Molecule, Plasmid, mRNA, or Minicircle etc.) in 500 µl of complete Blood Cell Culture Medium to the wells to induce reprogramming of PBMCs.

Note: Sendai Virus from Life Technologies Inc. is strongly recommended, Cat# A16517



3. Day 1: Wash the cells with Blood Cell Basal Medium (Stemmera, Cat # ST01005-S4/BM) to erase the reprogramming solution.
4. Add 500 µl-1ml of Blood Cell Basal Medium (Stemmera, Cat # ST01005-S4/BM) into the cells, transfer all the cells and medium to 15 ml Falcon tube and centrifuge the cells at 1000 rpm for 3-5 minutes.
5. Resuspend the cells in complete Blood Cell Growth Medium (Stemmera, Cat # ST01005-S4) and replate the cells into Matrix coated 12-well or 6-well plate, culture the cells in a 37°C, 5% CO₂ incubator overnight.

Note: 5x10³ or 1x10⁴ per well of 12-well plate is preferred.
6. Day 2: Take off one day.
7. Day 3: Transfer the suspension cells into new well of matrix coated 12 well-plate, add 1ml of complete Blood Cell Culture Medium (Stemmera, Cat # ST01005-S4) into old well from Day 1 to continue culturing for back up.
8. Day 4-6: Switch the medium to Blood Cell Basal Medium without supplement (Stemmera, Cat # ST01005-S4/BM) into 2 wells (one well from Day 1, one well from Day 3). Change medium every other day.
9. Day 7-9: Switch the medium to 50% of Blood Cell Basal Medium (Stemmera, Cat # ST01005-S4/BM) and 50% of complete Blood Cell Reprogramming Medium (Stemmera, Cat # ST01005-S2)
10. Day 10+: Switch the medium to 100% complete Human Blood Cell Reprogramming Medium. Change medium daily until the colonies picked up.
11. On day 20-30: pick the colonies into Matrix coated 12-well plate with **complete Human Blood Cell Reprogramming Medium** with 10nM of Rock Inhibitor (Stemmera, Cat # ST01005-S7, 1:1000 dilution) and culture overnight. Change medium to regular complete **Human Blood Cell Reprogramming Medium** without Rock inhibitor the following day.
12. Keep culturing the colonies in complete **Human Blood Cell Reprogramming Medium** until the colonies ready to be split.
13. When the picked colonies reach 80-90% confluency (generally take 4-5 days), aspirate spent medium from the wells and discard.
14. Add 1 ml of Non-Enzymatic Cell Dissociation Solution (Stemmera, Cat # ST01005-S1) to each well. Ensure complete coverage of cell monolayer. Incubate for 5 minutes in a 37°C, 5% CO₂ incubator.
15. Observe cells periodically under an inverted microscope until the cells begin to round up. (Note: avoid cells from detached completely).
16. Gently aspirate and discard the Cell Dissociation Solution from the wells. Add 1 ml of complete Human Blood Cell Reprogramming Medium in each well and use a 1 ml pipette to gently pipet cells up and down a few times in well to further break up cell colonies until getting the single cells. (Note: Pipet carefully to reduce foaming).
17. Transfer the cells into a new well of Matrix coated 6-well plate and add sufficient complete medium with 10nM of Rock Inhibitor (Stemmera, Cat # ST01005-S7, 1:1000 dilution) (split ratio is 1:6-1:12).
18. Incubate the cells in a 37°C, 5% CO₂ incubator overnight.
19. Change medium to complete Human Blood Cell Reprogramming Medium without Rock Inhibitor the following day and change medium daily until cells reach 80-90% confluency and subculture the cells for maintenance or cryopreserve cells in liquid nitrogen for long-term storage.

Cryopreserve stem cells with Stemmera™ Serum-Free Cryopreservation Solution for hPSC (Stemmera, Cat # ST01005-S9)

1. (Optional) Change medium 1-2 hours before freezing the cells.
2. Harvest cells by following steps 14 through 16 in **Subculture and maintenance of the colonies in complete Human Blood Cell Reprogramming Medium.**
3. Transfer 1 ml of single cell suspension into 15 ml Falcon tube.
4. Centrifuge tube at 1000 rpm for 5 minutes.
5. Aspirate and discard supernatant. Take extreme care not to disturb the cell pellet. Calculate the volume of cryopreservation solution required to give a cell density of 2x10⁶ viable cells/ ml.
6. Resuspend the pellet with Serum-Free Cryopreservation Solution (Stemmera, Cat # ST01005-S9) at accurate volume and aliquot the cells to cryovials (1 ml/ vial).

Subculture and maintenance of the colonies in complete Human Blood Cell Reprogramming Medium

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7. Achieve cryopreservation in an automated or manual controlled rate freezing apparatus following standard procedures (1°C decrease per minute).
8. Transfer frozen cells to liquid nitrogen. We recommend vapor phase storage at -200°C to -150°C for a few years.

Quality Control

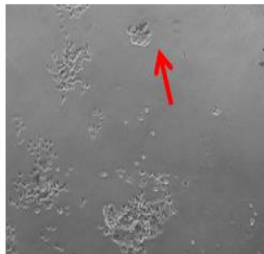
This product is used to reprogram human PBMCs to iPSCs and maintain the pluripotency of human iPSCs under serum-/feeder-free culture condition. To ensure the quality, the following images represent human PBMCs in suspension and the pluripotent morphology of human iPSCs growing on Matrigel with Stemmera™ Human Blood Cell (hBLD) Reprogramming Medium.



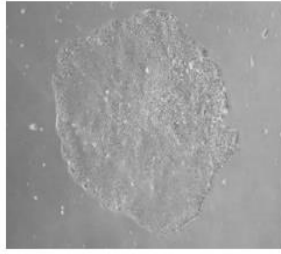
Human PBMCs in suspension



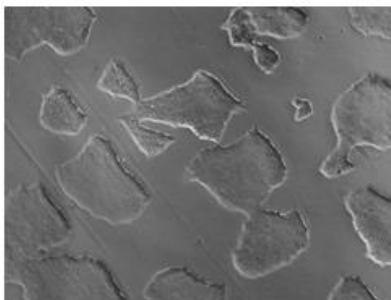
Day 1 post-transduction



Day 10 post-transduction



Day 26 post-transduction



Reprogrammed human iPSCs
after picking

Related Products

Product	Cat #	Size
Alkaline Phosphatase Detection Kit (Ready-to-Use) - Blue	ST01001	50 tests
Alkaline Phosphatase Detection Kit (Ready-to-Use) - Red	ST01002	50 tests
Human Fibroblast (hFIB) Reprogramming Kit	ST01003	5 reactions
Cell Karyotyping Analysis Kit	ST01004	20 reactions
Human ESCs/iPSCs Serum-/Feeder-free Medium (hStemSFM)	ST02001	500 ml
Human ESCs/iPSCs Xeno-Free Medium (hStemXFM)	ST02002	500 ml
Human Fibroblast (hFIB) Reprogramming Medium	ST02003	500 ml
Human Blood Cell (hBLD) Reprogramming Medium	ST02004	500 ml
Fibroblast Growth Medium	ST02005	500 ml
Human Blood Cell (hBLD) Growth Medium	ST02006	2 x 25 ml
Non-Enzymatic Cell Dissociation Solution (1x)	ST03001	100 ml
Serum-Free Cryopreservation Solution for Human ESCs/iPSCs	ST03002	50 ml
0.2% Gelatin	ST03003	100 ml
0.1% Gelatin	ST03004	100 ml
Universal Cryopreservation Solution (1x)	ST03005	50 ml
Blood Cell Cryopreservation Solution (1x)	ST03006	50 ml

Warranty and Limited Liability

Stemmera™ will not be liable for any damage caused by misuse, improper handling and storage of the product, non-compliance with precautions and procedures, and damages caused by events occurring after the product is released.

Technical Support

For more product and technical information, please visit our website at www.stemmera.com.

For further assistance, email your inquiries to our Technical Support team at techsupport@stemmera.com

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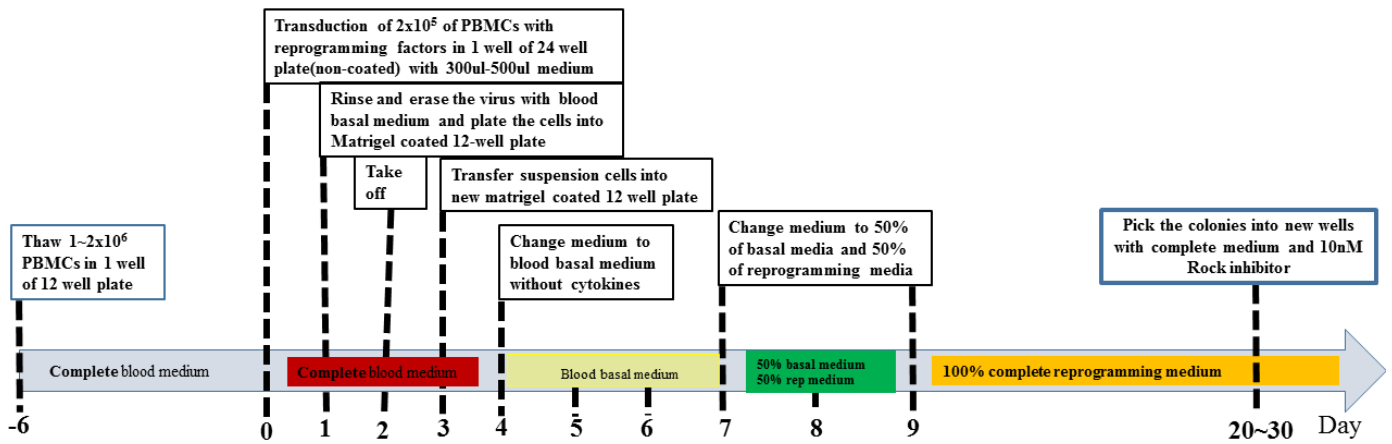
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Human Blood Cell Reprogramming Timeline



Rev. No.: 1.2
Date: Apr 12th, 2022