BMEM Human Primed Pluripotent –
Optimized maintenance medium for human iPSC

Ships at room temperature

Catalog number: 1001 **1 kit**

Product Description

BMEM Human Primed Pluripotent, comprising of BMEM Basal Medium and Primed Supplement, is a highly optimized, chemically defined, animal component-free basal medium and supplement combination for the maintenance and expansion of human induced pluripotent stem cells (hiPSCs) in adherent culture.

BMEM Basal Medium is based on the rigorously optimized published formulation[1].

Primed Supplement is new supplement specifically optimized for compatibility with adaptation of hiPSCs from legacy media such as mTeSR1™, a common problem with chemically defined media. Primed Supplement is also optimized for even further component stability and to allow room temperature shipment.

Culture in BMEM Human Primed Pluripotent results in increased cell proliferation and upregulation of core primed pluripotency factors when compared to legacy formulae[1]. BMEM Human Primed Pluripotent is formulated for compatibility with weekend-free and no-change feeding schedules protocols.

BMEM Human Primed Pluripotent is compatible with a variety of culture matrices, including Corning® Matrigel® GFR (354230), Gibco™ Geltrex™ GFR (A1413202), R&D Systems™ Cultrex™ UltiMatrix GFR (BME001-05), SigmaAldrich® ECMatrix™-511 E8 Laminin (CC160), Gibco™ Recombinant Vitronectin (A14700), Corning® Synthemax II-SC (3535).

BMEM Human Primed Pluripotent is compatible with a variety of passaging methods including BMEM Dissociation (1002), Gibco™ TrypLE™ (12604021), Stemcell Technologies™ ReLeSR™ (100-0483), and Gibco™ Versene (15040066).

Each lot of BMEM Basal Medium and Primed Supplement is performance tested on hiPSC and sterility tested.

COMPONENT NAME	SIZE	STORAGE
BMEM Basal Medium	500 mL	Store at 4°C
Primed Supplement	Lyophilized powder, vial	Store at -20°C

Instructions for Use

Use sterile technique to prepare complete BMEM Human Primed Pluripotent (basal medium + supplement). Complete BMEM can be used straight from the refrigerator (4 °C) and should not be warmed to RT or 37 °C prior to use.

1. Add 1 mL of BMEM Basal Medium to the vial of Primed Supplement, wait 3-5 minutes and pipette up and down to resuspend. Transfer the resuspended powder to the BMEM Basal Medium and wash out vial with BMEM. Gently mix by inversion.
2. Store complete BMEM at 4 °C for up to 30 days.

Directions for Use

Incubator atmosphere: Humidified atmosphere of 5% CO_2. Hypoxia (5% O_2) can be used but is not essential. Ensure that proper gas exchange is achieved in culture vessels.

BMEM can be used like any other traditional hiPSC medium. It is recommended to passage cells with BMEM Dissociation when they reach 70-80% confluence, split ratios should be modified to achieve this level of confluence every 4 days, selecting the highest split ratio possible. Cells should be passaged every 5 days irrelevant of confluence, adjusting the split ratio to compensate. Once adapted to BMEM, which may take 5-10 passages, cells will grow faster than in legacy media resulting in a requirement for higher split ratios. Consistency is beneficial for subsequent differentiation. Do not allow cells to become 100% confluent as it will result in slow growth and unreliable passaging.

ROCK Inhibitor

Addition of a ROCK inhibitor, such as 2 µM thiazovivin or 10 µM Y27632 for 24 hours after passage is recommended to provide more reproducible passaging, reduce selective pressure, and allow higher split ratios.

Feeding Schedule

Daily media changes provide optimal cell growth and should be used when adapting cells to BMEM. Once adapted, BMEM is compatible with day-skipping, weekend-free, and no-change protocols – all feeding schedules will maintain pluripotency as long as cells are split 70-80% confluence.

Adaptation from Legacy Media

BMEM has been specifically modified to ease the transition from legacy media. Spontaneous differentiation during transition should not be seen.

The morphology of cells in chemically defined media such as BMEM differs from that seen in mTeSR1™, mTeSR™ Plus, and StemFlex™. Cells in BMEM exhibit a looser morphology and do not grow as colonies but as looser non-colony ‘continents’ which will become a monolayer if allowed to overgrow. It is common for cells transitioning from the tight mTeSR1™ morphology to the loose chemically defined morphology to be mistaken for spontaneous differentiation. This is not the case, spontaneous differentiation will not be seen in BMEM cultured cells and no ‘picking’ should be expected.

To Adapt Cells from Legacy Media

1. Passage confluent cells using your normal dissociation reagent into BMEM with a ROCK inhibitor at a 1:5, 1:10, and 1:15 ratio.

2. Change media daily.
3. After 4 days (or 5 days if confluence is very low) choose the well with the highest split ratio that achieved 70-80% confluence. Passage these cells at a 1:5, 1:10, and 1:15 ratio.
4. Keep repeating this ratio testing process until you settle on a split ratio that achieves 70-80% confluence after 4 days (typically 5-10 passages) increasing the ratios assessed (i.e. 1:15, 1:20, 1:25) as cells adapt.

NOTE: The aim is to eliminate the mTeSR1-type dense colony growth. Switching media mid-passage or using 50:50 mixes of media results in slower adaptation.

Product Use

For Research Use Only.

Not for diagnostic procedures.

Reference

1. Lyra-Leite, D.M., Copley, R.R., Freeman, P.P., Pongpamorn, P., Shah, D., McKenna, D.E., Lenny, B., Pinheiro, E.A., Weddle, C.J., Gharib, M., et al. (2023). Nutritional requirements of human induced pluripotent stem cells. Stem Cell Reports *18*, 1371-1387. 10.1016/j.stemcr.2023.05.004.

Troubleshooting

1. Slow growth and low confluency levels by day 4.
   - Cause: Split ratio was too high.
     - Solution: Obtain new cells and split more gently at a variety of ratios.
   - Cause: Media is too old.
     - Solution: Complete media should be used within 30 days.
   - Cause: Cells were either overconfluent (>90%) or underconfluent (<60%).
     - Solution: Adjust split ratio of hiPSCs to achieve 70-80% confluency after 4 days of growth.

2. hiPSCs are not attaching.
   - Cause: No extracellular matrix on the plates or matrix may be too old and have dried out.
     - Solution: Discard old plates and replace.
   - Cause: Cells were overconfluent before passaging and became contact inhibited.
     - Solution: Adjust split ratio of pluripotent cells to achieve 70-80% confluency after 4 days of growth.
   - Cause: Cells were kept in dissociation solution for too long.
     - Solution: Follow the specific dissociation times for each dissociation reagent.