Mouse Embryonic Fibroblast-Conditioned Medium (MEF-cm)

Catalog #5881

Product Description

Mouse Embryonic Fibroblast-Conditioned Medium (MEF-cm) is a serum free medium for the feeder independent culture of human pluripotent stem cells (hPSCs), including embryonic stem cells and induced pluripotent stem cells. MEF-cm is an efficient alternative to MEF feeder cells, supporting the self-renewal of hPSC and maintaining pluripotency in the presence of basic FGF and hPSC qualified matrix (e.g., BD Matrigel™ matrix).

ScienCell's MEF-cm was prepared using high quality mitomycin C treated MEF (MEF-mt, Cat. #M7570-mt) derived from E13 embryos of CF1 mice. Serum free medium was conditioned with mitomycin C treated CF1 MEFs for 24 hours. The conditioned medium was collected daily for 7 days, pooled and sterilized by filtering through a 0.22 µm filter.

Before using MEF-cm for hPSC culture, please add fresh recombinant human basic FGF (4 ng/mL) (rhbFGF, Cat. #104-02) into the medium to make the complete medium. The complete MEF-cm has been tested for its ability to support hPSC self-renewal on BD Matrigel™ matrix *in vitro*. The medium color of each lot may vary but does not affect performance.

Components

MEF-cm consists of 100 mL of the medium in two 50-mL bottles.

Additional Materials Required

The recombinant human basic FGF (rhbFGF, Cat. #104-02) should be purchased separately.

Product Use

MEF-cm is for research use only. It is not approved for human or animal use, or for application in *in vitro* diagnostic procedures.

Storage

Store MEF-cm at ≤ -20°C in a manual defrost freezer. Thaw the medium overnight at 2-8°C in the dark. Aliquot and store the unused portions at ≤ -20°C. Long-term storage (>2 weeks) at 2-8°C is not recommended. Avoid repeated freeze-thaw cycles.

Shipping

Dry ice.

Mouse Embryonic Fibroblast-Conditioned Medium Preparation

1. One day before using, take out MEF-cm from the ≤ -20°C freezer. Thaw the medium overnight at 2-8°C in the dark.

2. Decontaminate the external surfaces of the medium bottle and tube containing rhFGF-basic with 70% ethanol and transfer them to a sterile field.

3. Add the rhFGF-basic (final concentration: 4 ng/mL) into MEF-cm using sterile techniques and mix well. The complete MEF-cm is now ready for use.

NOTE: Store the complete MEF-cm in the dark at 4°C. We recommend warming the medium to room temperature prior to use.

Instructions for use

When transitioning hPSC from feeder culture to feeder- free MEF-cm culture, no adaptation step is required. Simply replate the hPSC aggregates in MEF-cm into Matrigel™-coated vessels. We recommend keeping a culture using the previous culture system in parallel.

1. Prepare BD Matrigel™-coated culture vessel according to the manufacturer's instructions and warm to room temperature prior to use.

2. Before passaging, under a microscope mark the differentiated hPSC colonies and remove them by scraping in a sterile field.

3. Wash the culture with DMEM/F12 basal medium to remove any scraped colonies.

4. Use preferred methods to dissociate the remaining un-differentiated hPSC colonies and break them into cell aggregates.

5. Pellet hPSC aggregates by centrifuging at 1,000x rpm for 5 minutes. Resuspend cell aggregates in MEF-cm using a 2-mL pipette by gently pipetting up and down 1-2 times. Avoid excessive pipetting, which will generate single cells.
   Note: It is critical to passage hPSC as cell aggregates to maintain pluripotency and good viability.

6. Replate hPSC aggregates in MEF-cm into Matrigel™-coated vessels at an appropriate density. Change fresh MEF-cm daily for the cells.

Caution: If handled improperly, some components of the medium may present a health hazard. Take appropriate precautions when handling it, including the wearing of protective clothing and eyewear. Dispose of properly.