



**bFGF-Stimulated Mouse Embryonic Fibroblast-Conditioned Medium
(bFGF-std MEF-cm)**
Catalog #5891

Product Description

bFGF-Stimulated Mouse Embryonic Fibroblast-Conditioned Medium (bFGF-std MEF-cm) is a complete serum-free medium for the feeder-free culture of human pluripotent stem cells (hPSCs), including embryonic stem cells and induced pluripotent stem cells. bFGF-std MEF-cm is a complete medium containing recombinant human basic FGF (rhbFGF) and is ready to use after thawing, so there is no need to add extra basic FGF. It supports the self-renewal of hPSC and maintains pluripotency in the presence of hPSC qualified matrix (e.g., BD Matrigel™ matrix).

bFGF-std 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. Recombinant human basic FGF (rhbFGF, Cat. #104-02) was added to the medium at a final concentration of 4 ng/mL to make the complete medium.

bFGF-std 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.

Component

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

Product Use

bFGF-std 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 bFGF-std MEF-cm at $\leq -20^{\circ}\text{C}$ in a manual defrost freezer. Thaw the medium overnight at $2-8^{\circ}\text{C}$ in the dark. Long-term storage (>2 weeks) at $2-8^{\circ}\text{C}$ is not recommended. Avoid repeated freeze-thaw cycles.

Shipping

Dry ice.

bFGF-std Mouse Embryonic Fibroblast-Conditioned Medium Preparation

1. One day before using, take out bFGF-std MEF-cm from a $\leq -20^{\circ}\text{C}$ freezer. Thaw the medium overnight at $2-8^{\circ}\text{C}$ in the dark.
2. On the next day, warm up the medium to room temperature.
3. Decontaminate the external surface of the medium bottle with 70% ethanol and transfer it to a sterile field. Now it is ready for use.

NOTE: Store the thawed bFGF-std MEF-cm in the dark at 4°C .

Instructions for use

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

1. Prepare BD MatrigelTM-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 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 bFGF-std 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 bFGF-std MEF-cm into MatrigelTM-coated vessels at an appropriate density. Change fresh medium 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.