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STEMmatrix™ BME

hPSC-qualified basement membrane extract that supports feeder-free expansion and differentiation of hPSCs

Catalog #200-0960 5 mL

Product Description

STEMmatrix™ BME is a soluble basement membrane matrix extracted from mouse tumors. STEMmatrix™ BME supports the feeder-free expansion of human pluripotent stem cells (hPSCs). The main extracellular matrix components of STEMmatrix™ BME include collagen IV, entactin, heparan sulphate proteoglycans, and laminin. The growth factor components include epidermal growth factor (EGF), fibroblast growth factor (bFGF), insulin-like growth factor (IGF-1), transforming growth factor-beta (TGF-β), and vascular endothelial growth factor (VEGF).

STEMmatrix™ BME is hPSC-qualified and has been used to successfully maintain hPSC lines in the undifferentiated state when the cells are cultured in feeder-free hPSC maintenance media, such as mTeSR™ Plus (Catalog #100-0276), eTeSR™ (Catalog #100-1215), mTeSR™ (Catalog #85850), or TeSR™-E8™ (Catalog #05990). These hPSCs retain the characteristic morphology and expression of undifferentiated cell markers, such as OCT4 and TRA-1-60.

Properties

Stability and Storage:	Refer to the expiry date on the Certificate of Analysis (CoA), available at www.stemcell.com/coa. Store at -20°C. Avoid repeated freezing and thawing.
Contains:	Supplied in Dulbecco's Modified Eagle's Medium with 50 µg/mL gentamycin. Physical Appearance: Clear liquid

Directions for Use

The following instructions are for preparing 25 mL of STEMmatrix™ BME working solution at a final concentration of 100 µg/mL. This volume is sufficient for coating 4 x 6-well plates (1 mL/well) or 3 x 100 mm dishes (8 mL/dish). If using other cultureware, adjust volumes accordingly.

NOTE: Use tissue culture-treated cultureware (e.g. Falcon® 6-Well Flat-Bottom Plate, Tissue Culture-Treated; Catalog #38016) with STEMmatrix™ BME.

1. Thaw STEMmatrix™ BME by submerging the vial in ice and placing it at 2 - 8°C overnight.
   NOTE: It is recommended to prepare aliquots of STEMmatrix™ BME and store at -20°C until use. Do not exceed the shelf life of the product. After thawing aliquots, use immediately. Do not refreeze.
2. Swirl the vial to ensure that STEMmatrix™ BME appears evenly dispersed. Spray the top of the vial with 70% ethanol and air dry. Keep product on ice and handle using sterile technique.
   NOTE: Ensure that STEMmatrix™ BME, dilution medium, and all consumables are ice-cold. STEMmatrix™ BME starts to gel above 4°C. Keep on ice when thawing and handling to avoid gelling. To liquefy, submerge the vial in ice for 1 - 2 hours.

3. Calculate the required volume of STEMmatrix™ BME to achieve a final concentration of 100 µg/mL in 25 mL of STEMmatrix™ BME working solution.
   NOTE: For the lot-specific protein concentration, refer to the CoA, available at www.stemcell.com/coa.
   Example: If protein concentration of STEMmatrix™ BME is 9.8 mg/mL, adding 255.1 µL to 24.75 mL of dilution medium achieves a final concentration of 100 µg/mL in 25 mL.
4. Aliquot STEMmatrix™ BME in pre-chilled tubes, according to the calculated volume in step 3. Switch pipette tips if clogged by STEMmatrix™ BME. Keep tubes on ice.
   NOTE: If not used immediately, store at -20°C. Do not exceed the shelf life of the product. Thaw aliquots on ice.
5. Prepare the diluted STEMmatrix™ BME working solution using the volumes calculated in step 3 and following the instructions below:
   i. Add cold dilution medium (DMEM/F-12 with 15 mM HEPES; Catalog #36254) to a 50 mL conical tube (e.g. Falcon® Conical Tubes, 50 mL Catalog #38010) and place on ice.
   ii. Add one aliquot of STEMmatrix™ BME to the cold dilution medium. Mix thoroughly.
6. Immediately add the diluted STEMmatrix™ BME working solution to cultureware. Gently rock the cultureware back and forth to spread the solution evenly across the surface.
   NOTE: If the cultureware surface is not fully coated by the STEMmatrix™ BME solution, it should not be used for hPSC culture.
7. Incubate at room temperature (15 - 25°C) for at least 1 hour before use. Do not let the solution evaporate.
   NOTE: If not used immediately, seal the cultureware to prevent evaporation of the STEMmatrix™ BME solution (e.g. with Parafilm®) and store at 2 - 4°C for up to 1 week. When ready to plate the cells, allow stored coated cultureware to warm to room temperature for 30 minutes before proceeding to the next step.
8. Gently tilt the cultureware to one side and allow the excess STEMmatrix™ BME working solution to collect at the edge. Remove excess STEMmatrix™ BME working solution using a serological pipette or by aspiration. Ensure that the coated surface is not scratched. Coated cultureware is now ready for use.

References

Bissell DM et al. (1987) Support of cultured hepatocytes by a laminin-rich gel. Evidence for a functionally significant subendothelial matrix in normal rat liver. J Clin Invest 79(3): 801.

Kleinman HK et al. (1982) Isolation and Characterization of Type IV Procollagen, Laminin, and Heparan Sulfate Proteoglycan from the EHS Sarcoma. Biochem 21(24): 6188–93.

Kleinman HK et al. (1986) Basement membrane complexes with biological activity. Biochem 25(2): 312–8.