STEMdiff™ Mesoderm Induction Medium

Defined, xeno-free induction medium for early mesodermal differentiation

Catalog #05220 100 mL
#05221 500 mL

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Product Description

STEMdiff™ Mesoderm Induction Medium is a defined, serum- and xeno-free medium for the generation of early mesoderm cells from human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells. Medium is complete as provided and does not require further addition of cytokines or other factors. Mesoderm induction is achieved using a short and simple monolayer protocol. STEMdiff™ Mesoderm Induction Medium has been optimized for the differentiation of human ES and iPS cells cultured in either mTeSR™1, TeSR™-E8™ medium, mTeSR™ Plus, or TeSR™-AOF. The purity of early mesoderm cells (Brachyury+OCT4-) obtained with STEMdiff™ Mesoderm Induction Medium is typically 80 - 99%.

Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
STEMdiff™ Mesoderm Induction Medium	05220	100 mL	Store at -20°C.	Stable until expiry date (EXP) on label.
STEMdiff™ Mesoderm Induction Medium	05221	500 mL	Store at -20°C.	Stable until expiry date (EXP) on label.

This product contains material derived from human plasma. Donors have been tested and found negative for HIV-1 and -2, hepatitis B, and hepatitis C prior to donation. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Materials Required but Not Included

PRODUCT NAME	CATALOG #
Corning® Matrigel® hESC-Qualified Matrix OR Vitronectin XF™	Corning 354277 OR 07180
DMEM/F-12 with 15 mM HEPES	36254
D-PBS (Without Ca++ and Mg++)	37350
Gentle Cell Dissociation Reagent	07174
mTeSR™1 OR TeSR™-E8™ OR mTeSR™ Plus OR TeSR™-AOF	85850 OR 05990 OR 100-0276 OR 100-0401
Trypan Blue	07050
Y-27632 (Dihydrochloride)	72302

Directions for Use

NOTE: For complete instructions on coating plates with Corning® Matrigel® or Vitronectin XF™, and maintaining high-quality human ES and iPS cells for use in differentiation, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #10000005505), TeSR™-E8™ (Document #10000005516), mTeSR™ Plus (Document #10000007757), or TeSR™-AOF (Document #10000008160), available at www.stemcell.com or contact us to request a copy. Matrix-coated plates should be prepared in advance and brought to room temperature (15 - 25°C) for at least 30 minutes prior to use.

Thaw STEMdiff™ Mesoderm Induction Medium at room temperature (15 - 25°C) or overnight at 2 - 8°C. Mix thoroughly.

NOTE: Do not filter medium. Once thawed, use immediately or store at 2 - 8°C for up to 1 month. Alternatively, aliquot into polypropylene or PET-E tubes or bottles and store at -20°C. After thawing aliquots, use immediately or store at 2 - 8°C for up to 2 weeks. Do not re-aliquot into additional tubes or bottles.

Use sterile technique when performing the following protocols. The following are instructions for use with 6-well plates. Indicated volumes are for a single well. If using alternative cultureware, adjust volumes accordingly.

A. PLATING CELLS FOR MESODERM INDUCTION

NOTE: Human ES and iPS cells are ready for passage when the majority of colonies are large, compact, and have centers that are dense compared to their edges.

1. On Day 0, bring to room temperature (15 - 25°C) sufficient volumes of the appropriate medium (mTeSR™1, TeSR™-E8™, mTeSR™ Plus, or TeSR™-AOF), DMEM/F-12, and Gentle Cell Dissociation Reagent for passaging.
2. Prepare Single-Cell Plating Medium by adding Y-27632 to the medium used for cell maintenance (e.g. mTeSR™1, TeSR™-E8™, mTeSR™ Plus, or TeSR™-AOF) to reach a final concentration of 10 µM.
3. Wash the well to be passaged with 1 mL of D-PBS (Without Ca++ and Mg++).
4. Aspirate the wash medium and add 1 mL of Gentle Cell Dissociation Reagent.
5. Incubate at 37°C for 8 - 10 minutes.
6. Harvest cells by pipetting up and down with either a serological pipette (e.g. Catalog #38001) or a 1 mL pipette to ensure a single-cell suspension and transfer cells to a 15 mL conical tube (e.g. Catalog #38009) containing an equal volume of medium (DMEM/F-12, mTeSR™1, TeSR™-E8™, mTeSR™ Plus, or TeSR™-AOF). Rinse wells with an additional 1 - 2 mL of medium and add the rinse to the tube containing the cells.
7. Centrifuge cells at 300 x g for 5 minutes.
8. Resuspend cells in 1 mL of Single-Cell Plating Medium and perform a viable cell count using Trypan Blue and a hemocytometer.
9. Plate cells at a density of 5 x 10^4 cells per cm^2 onto coated plates in 2 mL of Single-Cell Plating Medium. The recommended number of cells for various plate sizes is as follows:
   - 6-well plate: 5 x 10^5 cells/well
   - 12-well plate: 2 x 10^5 cells/well
   - 24-well plate: 1 x 10^5 cells/well

   Adjust cell density if necessary, so that the cells are between 20 - 50% confluent on Day 1.

   If seeding cells for downstream diffrentiation with STEMdiff™ Endothelial Differentiation Kit (Catalog #08005), seed cells at a density of 0.5 - 1 x 10^4 cells/cm^2 in mTeSR™1 + 10 µM Y-27632 on a Matrigel®-coated 6-well plate.
   NOTE: The seeding density may need to be adjusted depending on the cell line and maintenance medium used. Refer to Table 2 of the Product Information Sheet (Document #10000006932) for recommended plating densities.
10. Incubate at 37°C for 24 hours.
11. Continue to section B (Differentiating Monolayer Cultures to Mesoderm).

B. DIFFERENTIATING MONOLAYER CULTURES TO MESODERM

1. On Day 1, warm the bottle of STEMdiff™ Mesoderm Induction Medium to room temperature (15 - 25°C) before use.
2. Aspirate medium from the well and replace with STEMdiff™ Mesoderm Induction Medium. Recommended volumes are as follows:
   - 6-well plate: 3 mL/well
   - 12-well plate: 1.5 mL/well
   - 24-well plate: 1 mL/well
3. Incubate at 37°C for 24 hours.
4. On Days 2 - 4, repeat steps 1 - 3 above.

5. On Day 5, cells are ready to be assayed for the formation of early mesoderm or carried forward into more specialized lineage differentiation protocols.

NOTE: The optimal timing for transfer into downstream differentiation conditions may vary for different specialized cell types. Cells may be carried forward into more specialized lineage differentiation protocols between Days 3 - 5.

C. ASSESSMENT OF EARLY MESODERM CELLS

Purity of early mesoderm cells can be measured by flow cytometry after labeling with fluorochrome-conjugated anti-Brachyury antibody or anti-NCAM (e.g. Anti-Human CD56 [NCAM] Antibody, Clone HCD56, Catalog #60021). The absence of undifferentiated cells can be confirmed by flow cytometry after labeling with fluorochrome-conjugated anti-OCT4 (e.g. Anti-Human OCT4 [OCT3] Antibody, Clone 3A2A20, Catalog #60093). Results may vary depending on cell line used.

Related Products

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