Applied StemCell, Inc.
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Datasheet

Stromal Cells Culture Protocols

Product Information

Catalog Number **ASM-6004 (Stromal Cellulations Medium)**

Materials Required

Materials	Equipment
• Cells ASE-5005, ASE-5006, ASE-5007, ASE-5194^5196 • 1X PBS (Ca2+/Mg2+-free) • Stromal Cellutions Media (ASM-6004) Collagen coating solution (150µg/mL) • Dimethyl Sulfoxide (DMSO) • Fetal Bovine Serum (FBS) • TrypLE Express • 15.0 & 50.0 mL tubes • Tissue culture vessels or 6 well plates • 2-1000µl Pipets • 2-100µl Pipet Tips • Pipet Aid • Serological Pipet Tips • Mr. Frosty • Ice	• Incubator, 37°C/5% CO2 • Water Bath 37°C • Centrifuge • Pipette

Protocol

1. **Immediately Upon Delivery**
   - 1.1 Remove vial from shipping container to check that it is still frozen.
   - 1.2 Transfer frozel vial to liquid nitrogen until you are ready to thaw and begin cell culture.

2. **Thawing Cells**
   - 2.1 Prepare complete Stromal Cellutions Media (can be stored at 4°C for a maximum of 30 days).
   - 2.2 Aliquot and warm only media required.
   - 2.3 Thaw cells rapidly and with agitation (See Hot to Thaw Cells).
   - 2.4 Centrifuge at 400 g for 5 minutes. Remove supernatant and resuspend cell pellet in 1-5 mL fresh 37°C warm Stromal Cellutions Media.
   - 2.5 Count cells (see How to Count Fresh Cells).

3. **Cultivating Cells**
   - 3.1 Prepare an aliquot of cells for plating at a density:
     - 1.0-5.0 x 10^4 cells/cm2 for mononuclear cells
     - 1.0 x 10^3 cells/cm^2 for stromal cells.
   - 3.2 Gently mix warm media with cells for plating.
   - 3.3 Carefully add cell suspension prepared to flasks/plate at correct seeding density.
   - 3.4 Place flasks/plates in a CO^2 incubator.
     - For mononuclear cells, let cells sit undisturbed for no less than 3-4 days, change media on the 5th day.
     - For Stromal Cells, proceed to next step.

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Note: You will lose a lot of non-adherent mononuclear cells at this point. See note below for continuing with non-adherent cells.

- *Feed cells every 2-3 days by performing a complete media change as follows:*
- *Aspirate Media*
  - *Add warm PBS*
  - *Aspirate PBS*
  - *Add fresh media*

Note: Mononuclear cells will have the vast majority of the cells floating. They will not adhere. Collect the supernatant if you are interested in investigating other cell types besides stromal.

- *Visualize cells every 2-3 days.*
- *Once cells become 70% confluent prepare cells for passage, collection, or freezing.*

Note: Mononuclear cells will take 3-4 days to attach and may take up to 3 weeks to reach 70% confluent.

Note: Do not let mononuclear cells grow to more than 70% confluency or for > 14 days.

4. **Passing and Sub-cultivation of Cells**

4.1 Aspirate and discard medium; wash cells with PBS (Ca2+/Mg2+ free); add TrypLE enzyme solution or similar and incubate for 5-10 min at 37°C. (for details see How to Passage Cells).

Note: Visualize cells and collect as soon as they are lifted off the plate. DO NOT over trypsinize.

4.2 Transfer all enzyme solution mixture into a conical tube containing 10% volume of cold H-GRO or FBS, keep it chilled on ice.

4.3 Wash flasks/plate with equal volume PBS and collect any remainder cells

4.4 Centrifuge the collected cell mixture in the conical tube at 400 g for 5 minutes, and aspirate supernatant

4.5 If plating cells to another cell culture vessel:
- Resuspend cell pellet in pre-warmed media
- Perform cell count
- Plate cells at 1.0 x 10^3 cells/cm^2
- Refer to product information sheet for specifics on characterization of cells.

4.6 If freezing cells for storage:
- Resuspend cell pellet in cold FBS
- Remove aliquot and perform cell count
- Place cells on ice
- Determine how many vials to freeze (recommended 1.0x10^6 to no more than 5.0x10^6 in 1mL per vial)
- Slowly and dropwise add equal volume cold FBS containing 20% (v/v) dimethyl sulfoxide (DMSO). This achieves a final volume of 10% (v/v) dimethyl sulfoxide (DMSO).
- Immediately remove vials to Mr. Frosty and place at -80°C or control rate freezer.