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Stem Cell in vitro Differentiation

(from Kit Manual)

Osteogenesis Differentiation

1. Observe cell monolayer from basal cultures expanded in StemPro® MSC SFM, MesenPRO RS™ medium, or standard growth medium (DMEM+10% FBS) to ensure mid-log growth phase confluence (60–80%). Aspirate medium and floating cells from culture flask and discard.

2. Add 5–10 mL DPBS. Gently rinse cell monolayer.

3. Remove DPBS, add 5–7 mL of pre-warmed TrypLE™ Express to flask and completely coat culture surface. Incubate for 5–8 minutes at 36°C to 38°C or until cells have fully detached.

4. Gently pipet detached cells into a single cell solution and verify on inverted microscope.

5. Remove cell suspension from flask, transfer into a centrifuge tube, and pellet cells at 100 × g for 5–10 minutes.

6. Determine cell viability and total cell density using Trypan Blue Stain and an electronic (Coulter Counter) or manual (hemocytometer) cell counting method.

7. Resuspend the pellet in an appropriate volume of pre-warmed MSC Growth Medium.

8. Seed MSCs into culture vessels at 5 × 103 cells/cm2. For classical stain differentiation assay, seed into a 12-well plate. For gene expression profile studies, seed into a T-75 flask. For immunocytochemistry studies, seed into a 16-well CultureWell™ chambered coverglass or 96-well plate.

9. Incubate the cells in MSC Growth Medium at 36°C to 38°C in a humidified atmosphere of 4-6% CO_2 for minimum of 2 hours up to 4 days.

10. Replace media with pre-warmed Complete Osteogenesis Differentiation Medium and continue incubation. MSCs will continue to expand as they differentiate under osteogenic conditions. Refeed cultures every 3-4 days.

11. After specific periods of cultivation, you can process osteogenic cultures for alkaline phosphatase staining (7-14 days) or Alizarin Red S staining (>21 says; see the following section for method), gene expression analysis, or protein detection.

Adipogenesis differentiation

1. Observe cell monolayer from basal cultures expanded in StemPro® MSC SFM, MesenPRO RS™ medium, or standard growth medium (DMEM+10% FBS) to ensure mid-log growth phase confluence (60–80%). Aspirate medium and floating cells from culture flask and discard.

2. Add 5–10 mL DPBS. Gently rinse cell monolayer.

3. Remove DPBS, add 5–7 mL of pre-warmed TrypLE™ Express to flask and completely coat culture surface. Incubate for 5–8 minutes at 36°C to 38°C or until cells have fully detached.

4. Gently pipet detached cells into a single cell solution and verify on inverted microscope.

5. Remove cell suspension from flask, transfer into a centrifuge tube, and pellet cells at 100 × g for 5–10 minutes.

6. Determine cell viability and total cell density using Trypan Blue Stain and an electronic (Coulter Counter) or manual (hemocytometer) cell counting method.

7. Resuspend the pellet in an appropriate volume of pre-warmed MSC Growth Medium.

8. Seed MSCs into culture vessels at 1 x 10^4 cells/cm^2. For classical stain differentiation assay, seed into a 12-well plate. For gene expression profile studies, seed into a T-75 flask. For immunocytochemistry studies, seed into a 16-well CultureWell™ chambered coverglass or 96-well plate.

9. Incubate the cells in MSC Growth Medium at 36°C to 38°C in a humidified atmosphere of 4-6% CO_2 for a minimum of 2 hours up to 4 days.

10. Replace media with pre-warmed Complete Adipogenesis Differentiation Medium and continue incubation. MSCs will continue to undergo limited expansion as they differentiate under adipogenic conditions. Refeed cultures every 3-4 days.

11. After specific periods of cultivation, adipogenic cultures can be processed for Oil Red O or LipidTOX™ staining (beginning at 7-14 days), gene expression analysis, or protein detection.

Chondrogenesis Differentiation

1. Observe cell monolayer from basal cultures expanded in StemPro® MSC SFM, MesenPRO RS™ medium, or standard growth medium (DMEM+10% FBS) to ensure mid-log growth phase confluence (60– 80%). Aspirate medium and floating cells from culture flask and discard.

2. Add 5–10 mL DPBS. Gently rinse cell monolayer.

3. Remove DPBS, add 5–7 mL of pre-warmed TrypLE™ Express to flask and completely coat culture surface. Incubate for 5–8 minutes at 36°C to 38°C or until cells have fully detached.

4. Gently pipet detached cells into a single cell solution and verify on inverted microscope.

5. Remove cell suspension from flask, transfer into a centrifuge tube, and pellet cells at 100 × g for 5–10 minutes.

6. Determine cell viability and total cell density using Trypan Blue Stain and an electronic (Coulter Counter) or manual (hemocytometer) cell counting method.

7. For MesenPRO™ RS expansion cultures, resuspend the pellet in an appropriate volume of pre-warmed MesenPRO™ RS media to generate a cell solution of 1.6 × 107 viable cells/mL. For StemPro® MSC SFM or standard growth medium, use MSC Attachment Medium to generate a cell solution of 1.6 × 107 viable cells/mL.

8. Generate micromass cultures by seeding 5-µL droplets of cell solution in the center of multi-well plate wells for classical stain or 100-mm Petri dish for gene expression analysis, protein detection, or immunohistochemistry.

9. After cultivating micromass cultures for 2 hours under high humidity conditions, add warmed chondrogenesis media to culture to culture vessels and incubate in 37°C incubator with 5% CO_2.

10. Refeed cultures every 2-3 days.

11. After specific periods of cultivation, you can process chondrogenic pellets for Alcian Blue or Safranin O staining (>14 days), gene expression analysis, protein detection, or immunohistochemistry.

(References)

1. N-Cadherin-Expressing Bone and Marrow Stromal Progenitor Cells Maintain Reserve Hematopoietic Stem Cells. Zhao M, Tao F, Venkatraman A, Li Z, Smith SE, Unruh J, Chen S, Ward C, Qian P, Perry JM, Marshall H, Wang J, He XC, Li L. *Cell Rep*. 2019;26:652-669 e656.

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