Human Mesenchymal Stem Cell Protocol: Oil Red O Staining of Adipogenic Cultures

Protocol SC 00011

For research use only

Background

Oil Red O staining is an assay performed to stain induced adipogenic cultures to detect mature adipocytes.

Required Materials

- Induced Adipogenic Cultures
- Thermo Scientific HyClone ES-Qualified DPBS (SH30850.03)
- 10% Formalin solution, neutral buffered (Fisher SF98)
- Oil Red O (Fisher M312512)
- 99% Isopropyl Alcohol
- DI water
- 60% Isopropyl Alcohol
- Hematoxylin (Fisher SH30-500D)
- Whatman conical filter paper (Fisher 09-845)
- Tap water

General Considerations

All procedures involving formalin must be done in a fume hood.
Take care to not leave the cells dry for more than 30 seconds though out this assay.
Gently add and remove all reagents indirectly to the monolayer to avoid cell detachment. For example, drip the reagent down the side of the culture plate.

Fixing Adipogenic Cultures

1. Remove cultures from incubator and place in a fume hood.
2. Remove media from the wells, always from the control first.
3. Gently rinse the plate with 2mL of sterile HyClone ES-Qualified DPBS.
4. Remove the DBPS and add 2mL 10% formalin. Incubate 30-60 minutes at room temperature.

Preparing Oil Red O Stain

1. Prepare a stock solution by weighing out 300mg of Oil Red O powder and adding this to 100 mL of 99% isopropanol. This stock solution is stable one year from the date it was made.
2. In the fume hood, mix 3 parts of Oil Red O stock solution with 2 parts DI water. Incubate 10 minutes at room temperature. This working solution is only stable for 2 hours.
3. Place a piece of Whatman filter paper in a funnel above a vessel.
4. Filter the Oil Red O working solution completely through the filter funnel.

Staining Adipogenic Cultures

1. Remove the formalin from each well and discard it according to your chemical waste disposal procedure.
2. Gently rinse each well with 2 mL DI water. Remove and discard as formalin waste.
3. Add 2 mL 60% isopropanol to each well and let sit for 5 minutes.
4. Remove isopropanol and add 2 mL Oil Red O working solution to each well, be sure to cover the entire monolayer. Incubate 5 minutes at room temperature.
5. Remove Oil Red O and rinse cultures with room temp tap water until the water rinses off clear.
6. Add 2 mL Hematoxylin stain into each well, be sure to cover the entire monolayer. Incubate 1 minute at room temperature.

7. Remove the hematoxylin and rinse the cultures with room temp tap water until the water rinses off clear.
8. Add 2 mL tap water to each well and view on a phase contrast microscope.
9. Lipids appear red and nuclei appear blue.

Related Protocols

- SC Protocol 00009 - Human Mesenchymal Stem Cell Protocol: Sub Culturing hMSCs
- SC Protocol 00010 - Human Mesenchymal Stem Cell Protocol: Adipogenic Differentiation

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