AdipoPlus (Adipogenesis Differentiation Medium)
For research use only

Catalogue number: BI-1101

Product Description

AdipoPlus is a ready-to-use medium developed for the in vitro adipogenesis differentiation of mesenchymal stem cells (MSCs) especially Human mesenchymal stem cells. Differentiated cells can be analyzed for lipid synthesis after about 3 weeks with Oil-Red-O staining. AdipoPlus is an excellent research tool to study adipocyte differentiation, adipogenesis and adipocyte related metabolic diseases. The medium contains all reagents for induction of adipogenesis pathways in MSCs to generate adipocytes.

Passaging and preparation of MSCs

1. Observe cell monolayer from basal cultures expanded in standard growth medium [DMEM (BI-1004) supplemented with 10% FBS (BI-1201) is recommended] to ensure mid-log growth phase confluence (60–80%). Aspirate medium and floating cells from culture flask and discard. Do not let passaged MSCs become completely confluent, as it can reduce the multipotency of MSCs.
2. Add 5–10 mL DPBS (BI-1401). Gently rinse cell monolayer.
3. Remove DPBS, add an adequate amount of pre-warmed trypsin from Bioidea (BI-1603 or BI-1604) to the flask and completely coat culture surface. Incubate for 5–8 minutes at 36°C to 38°C or until cells have fully detached. Overexpo sure to trypsin will lead to reduced MSC viability and expansion.
4. Gently pipet detached cells into a single cell solution and verify by inverted microscope.
5. Remove cell suspension from the flask, transfer to a centrifuge tube.
6. Pellet cells at 100 × g for 5–10 minutes.
7. Determine cell viability and total cell density using Trypan Blue Stain (BI-1803).
8. Re-suspend the pellet in an appropriate volume of pre-warmed MSC Growth Medium (DMEM + 10% FBS is recommended).
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. Inoculate plates at passage of T25 flask at approximately 1 x 10^4 cells/cm2 for low passage cells (strongly recommended), or 2 x 104 cells/cm^2 for high passage cells in standard growth medium (DMEM + 10% FBS is recommended). Continuously passaged MSCs will gradually lose their multipotency with increased passage number (>10 passages). Use one of the following quantities and plates:

- 2 ml growth medium per well for 2-well culture slides, OR
- 1 ml per well for 12-well plates, OR
- 0.5 ml per well for 24-well plates, OR
- 200 µl for 48-well plates.

Note

For subsequent spectrophotometric assay, leave triplicate wells empty for blanks, and triplicate wells of each ASC strain to be tested in non-induction medium

Adipogenesis differentiation

1. Examine cells every day to ensure mid-log growth phase confluence (60–80%), or are just confluent, remove complete growth medium and replace replicate wells with pre-warmed AdipoPlus Medium (BI-1101) and with control medium and continue incubation. MSCs will continue to undergo limited expansion as they differentiate under adipogenic conditions. Refeed cultures every 3 - 4 days. Expanding MSCs in growth medium for 2–4 days before refeeding with AdipoPlus Medium can enhance adipogenesis
2. After specific periods of cultivation, adipogenic cultures can be processed for Oil Red O (BI-1802) beginning at 7– 14 days, gene expression analysis, or protein detection.

   NOTE: Certain cell strains might continue to grow and become over-dense under these conditions, to the point that the cell monolayer clumps or lifts off.

   Some cultures may accumulate lipid more slowly than others. If the culture appears to be differentiating, but the putative lipid vesicles are still small after 2 weeks in AdipoPlus, the culture may be re-fed with fresh AdipoPlus and incubationcon tinued for another 4 days (21 days total in adipogenic conditions). Lipid droplets can be seen as early as 6 days incubated with differentiation medium.
3. The cells are then washed once with cold DPBS. If 2-well culture slides are used, aspirate the medium and add 2 ml cold DPBS per well, or 1 ml per well for 12-well and 24-well plates (use these volumes in all steps below as well.
4. Aspirate the wash and add Formaldehyde/ Calcium solution for a minimum of 10 minutes at room temperature, or a maximum of 24 hours at 4°C. If the Oil-Red-O staining cannot be done by this time, aspirate the fixative, replace with cold PBS, and store at 4°C for up to one week until ready to stain.

References

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