BI
Biological Industries

MSCgo™ Adipogenic

Serum-free, xeno-free medium for the direct differentiation of human mesenchymal stem cells into adipocytes

Instructions for Use

Medium Components

Product Description	Storage	Cat. No.	Size
MSCgo™ Adipogenic Basal Medium	2-8°C	05-330-1B	100ml
MSCgo™ Adipogenic Supplement Mix I	-20°C	05-331-1-01	100µl
MSCgo™ Adipogenic Supplement Mix II	-20°C	05-332-1-15	1.5ml

Product Description

MSCgo™ Adipogenic is a serum-free (SF), xeno-free (XF) medium developed for the differentiation of human Mesenchymal stem cells (hMSC) into mature adipocytes. The medium is suitable for variety sources of hMSC (e.g. bone marrow, adipose tissue and umbilical cord tissue; hMSC-BM, hMSC-AT, hMSC-CT) .

Product Use

For human ex vivo tissue and cell culture processing applications.
It is not approved for human or animal use, or for application of in vitro diagnostic procedures.

Adipogenesis Results

Adipogenic differentiation of hMSC results in the formation of spherical cells accumulated with lipid droplets that can be detected by inverted microscope. The amount of differentiated cells can be vary using different hMSC (e.g. types, age and passage).

Complete Ready-To-Use Medium Preparation

1. Thaw MSCgo™ Adipogenic supplement mix I and II at room temperature (RT).
2. Add 0.1ml of supplement mix I and 1.5ml of supplement mix II into 100ml of MSCgo™ Adipogenic basal medium. By adding the supplement mixes into the basal medium a complete ready to use medium is achieved.
3. The complete medium is stable for 1 month at 2-8°C.

Notes:
No additional additives are required for the complete, ready-to-use medium.
Contains L-alanine L-glutamine.
Does not contain antibiotics.

Required Materials for Adipogenic Assay

MSCgo™ Adipogenic:

BI; 05-330-1 , 05-331-1 and 05-332-1

- MSC NutriStem® XF : BI; 05-200-1 and 05-201-1
- MSC Attachment solution XF : BI; 05-752-1
- Optional- Oil Red O.

Precautions and Disclaimer

1. Do not use if a visible precipitate is observed in the medium.
2. Do not use the media beyond the expiration date indicated on the product label.

Note: Always use proper aseptic technique and work in a laminar flow hood.

Adipogenic Differentiation Assay

Initial Seeding of hMSC for Adipogenic Assay

Note: When handling biohazard materials such as human cells, appropriate safety procedures should always be used and protective clothing and gloves should be worn.

1. Seed $6x10^4$ cells/well in 24-well plate ($3x10^4$ cells/cm^2) using 0.5ml/well of MSC NutriStem® XF BI; 05-200-1 and 05-201-1, on pre-coated plates (using MSC attachment solution BI; 05-752-1, diluted 1:100 in DPBS).

   Note: For any other cultureware, the appropriate volume should be adjusted.

2. Incubate the cells in CO_2 incubator (37°C, 5% CO_2).

Initial of Differentiation

1. After 24hr from cell's seeding, ensure that the cells reach about 80% confluence and change medium to MSCgo™ Adipogenic complete medium (0.5ml/well; 24w/p).

   Note: If the cells confluence is <80% continue culturing in MSC NutriStem® XF for one more day.

2. Incubate the cells in CO_2 incubator (37°C, 5% CO_2) for 14-21 days with the recommended medium replacement as follow.

Medium Replacement Methods According to the Type of hMSC

hMSC originating from different sources represent different multi-lineage differentiation potential. For example, hMSC-AT required shorter induction period (~6 days) in comparison to hMSC-BM and hMSC-CT (~10 days).

Note: For all the different types of hMSC, it is recommended to use the maintenance medium (MSC NutiStem® XF) after the induction period of adipogenesis.

The medium replacement protocol has been adapted to different types of hMSC (hMSC-AT, hMSC-BM and hMSC-CT).

Note: Avoid pipetting the medium directly on the adipocytes and pipette very carefully, since adipocytes are fragile.

hMSC-AT:
one cycle of differentiation/maintenance media

- 6-8 days with complete MSCgo™ Adipogenic medium. Change medium every 3-4 days.
- After the adipogenic induction, replace the MSCgo™ Adipogenic complete medium into maintenance medium: MSC NutriStem® XF BI; 05-200-1 and 05-201-1 for period of 3-4 days.

hMSC-BM:
one cycle of differentiation/maintenance media

- 8-12 days with complete MSCgo™ Adipogenic medium. Change medium every 3-4 days (0.5ml/well; 24w/p).
- After the adipogenic induction, replace the MSCgo™ Adipogenic complete medium into maintenance medium: MSC NutriStem® XF BI; 05-200-1 and 05-201-1 for period of 3-4 days.

hMSC-CT:
at least two cycles of differentiation/maintenance media

- 5-10 days with complete MSCgo™ Adipogenic medium. Change medium every 3-4 days (0.5ml/well; 24w/p).
- After the adipogenic induction, when the cells become circled and start floating, replace the MSCgo™ Adipogenic complete medium into maintenance medium: MSC NutriStem® XF (05-200-1, 05-201-1, BI) for period of 3-6 days. Change medium every 2-4 days.
- Repeat these cycles until mature adipocytes are observed (i.e. formation of lipid droplets).

The plate is now ready for evaluation of adipogenesis.
Oil Red O can be used for the adipogenesis evaluation.
For the staining procedure follow the instructions ahead.

Oil red-O Staining Protocol (Optional)

Oil Red-O solution is used to stain lipid droplets accumulated intracellulary which are an indication of mature adipocytes.

Preparation of Oil Red-O Staining Stock Solution

1. Dissolve 0.35g Oil Red-O in 100 ml of 2-propanol, >99.5%.
2. Filter through a 0.2 or 0.45 micron PTFE filter.
3. The solution is stable for one year (2-8°C).

Prepare a Fresh Oil Red-O Staining Working Solution

1. Mix 6ml of Oil Red-O stock solution with 4ml DDW.
2. Mix well and let stand for 10-20 minutes at RT.
3. Filter through a 0.2 or 0.45 micron PTFE filter.
4. The solution can be used for up to 2-3 hours.

Oil Red-O staining Procedure

1. Carefully remove the medium and gentle wash once with DPBS; BI cat # 02-023-1 (1ml/well; 24w/p).
2. Fixation: carefully remove DPBS and add 10% Formalin (4% Formaldehyde) to each well (1ml/well; 24w/p). Incubate at room temperature, for 30-60 min.
3. Remove Formalin and wash once with DPBS (1ml/well; 24w/p).
4. Remove DPBS and add Oil Red-O working solution (1ml/well; 24w/p).
5. Incubate at room temperature for 10-30 minutes.
6. Wash with DDW (1ml/well; 24w/p) until to alimentation of excessive dye .
7. The plate is now ready for visual inspection and/or image acquisition.

Semi- Quantification of Oil Red O Staining (Optional)

1. Elute the dye by addition of 2-propanol, >99.5% (0.5ml/well; 24w/p).
2. Incubate at room temperature for 1hr.
3. Pipette to ensure that all Oil Red O is in the solution.
4. Read the absorbance (O.D.) at 500nm. (2-propanol, >99.5% serves as blank).

Quality Control

MSCgo™ Adipogenic performance is tested for optimal differentiation of hMSC into adipocytes . Additional tests are: pH, osmolality, endotoxins and sterility tests.

Biological Industries Israel Beit Haemek Ltd.
Kibbutz Beit Haemek 2511500, Israel
T.+972.4.9960595 F.+972.4.9968896
Email: info@bioind.com
www.bioind.com