Instructions for Use

# MSCgo™ Adipogenic

Serum-Free, Xeno-Free Medium for the Direct Differentiation of Human Mesenchymal Stem Cells into Adipocytes


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

| | MSCgo™ Adipogenic Basal Medium | MSCgo™ Adipogenic Supplement Mix I | MSCgo™ Adipogenic Supplement Mix II |
|---|---|---|---|
| REF | 05-330-1B | 05-331-1-01 | 05-332-1-15 |
|  | 2 – 8°C | -20 to -10°C | -20 to -10°C |

# Contents

# 1 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 hMSC from a variety of sources (e.g. bone marrow, adipose tissue and umbilical cord tissue; hMSC-BM, hMSC-AT, hMSC-CT) .

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

# 2 Features

- Serum-free, xeno-free medium
- All required growth factors and supplements included
- Reliable differentiation to mature adipocytes
- Each lot is application tested
- Contains stable L-alanyl-L-glutamine
- Does not contain antibiotics
- Sterile

# 3 Intended Use and Safety

1. For research or further manufacturing use as ancillary material in manufacturing of cell, gene or tissue-based products
2. Not intended for in vitro diagnostics use or use as a medical device
3. Not intended for human in vivo applications
4. Do not use the medium if visible particles and | or precipitate are observed.
5. Do not use the medium beyond the expiration date indicated on the product label.
6. Do not use in case of change of color.
7. Maintain aseptic work conditions.
8. Do not use if there is any package leakage or any exposure to environment conditions as the sterility of the product might be compromised.
9. Refer to the Material Safety Data Sheet (MSDS) for hazard information.

# 4 Storage and Stability

- Store MSCgo™ Adipogenic Basal Medium at 2 – 8°C.
- Store MSCgo™ Adipogenic Supplement Mix I and II at -20 to -10°C.
- The complete medium is stable for 1 month at 2 – 8°C.
- Protect the medium and supplements from direct light.
- Shelf life: refer to product label for expiration date.

# 5 Required Materials for Adipogenic Assay

- MSCgo™ Adipogenic: 05-330-1, 05-331-1 and 05-332-1
- MSC NutriStem® XF: 05-200-1 and 05-201-1
- NutriCoat™ Attachment Solution: 05-760-1-15
- Optional: Oil Red O

# 6 Complete Ready-To-Use Medium Preparation

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

# 7 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 $6 \times 10^4$ cells/well in 24-well plate ($3 \times 10^4$ cells/cm$^2$) using 0.5 mL/well of MSC NutriStem® XF 05-200-1 and 05-201-1, on pre-coated plates (using NutriCoat™ Attachment Solution, 05-760-1-15, diluted 1:500 in saline).

   **NOTE** For any other cultureware, the appropriate volume should be adjusted.

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

**Initial of Differentiation**

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

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

2. Incubate the cells in a $CO_2$ incubator (37°C, 5% $CO_2$) for 14 – 21 days with the recommended medium change as described in the following section.

### Medium Change Methods According to hMSC Type

hMSC originating from different sources represent different multi-lineage differentiation potential. For example, hMSC-AT require 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 change 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 05-200-1 and 05-201-1 for a 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 (0.5 mL/well; 24 w/p).
- After the adipogenic induction, replace the MSCgo™ Adipogenic complete medium into maintenance medium: MSC NutriStem® XF 05-200-1 and 05-201-1 for a 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 (0.5 mL/well; 24 w/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 for a 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.

# 8 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 µm PTFE filter.
3. The solution is stable for one year (2 - 8°C).

**Prepare a Fresh Oil Red O Staining Working Solution**

1. Mix 6 mL of Oil Red O stock solution with 4 mL DDW.
2. Mix well and let stand for 10 - 20 minutes at RT.
3. Filter through a 0.2 or 0.45 µm 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 (Cat. No. 02-023-1); (1 mL/well; 24 w/p).

2. Fixation: carefully remove DPBS and add 10% Formalin (4% Formaldehyde) to each well (1 mL/well; 24 w/p). Incubate at room temperature, for 30 - 60 min.
3. Remove Formalin and wash once with DPBS (1 mL/well; 24 w/p).
4. Remove DPBS and add Oil Red O working solution (1 mL/well; 24 w/p).
5. Incubate at room temperature for 10 - 30 minutes.
6. Wash with DDW (1 mL/well; 24 w/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% (0.5 mL/well; 24 w/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 500 nm. (2-propanol, >99.5% serves as blank).

# 9 Quality Control

MSCgo™ Adipogenic performance is tested for differentiation of hMSC into adipocytes. Additional tests are: pH, osmolality, endotoxins and sterility tests. For full specifications, please check the lot specific Certificate of Analysis (CoA).

# 10 Quality Assurance

- Manufactured under ISO 13485 and ISO 9001 QMS and in compliance with applicable cGMP guidelines
- Manufactured under controlled environments and processes in accordance with:
  1. ISO 13408 – Aseptic processing of health care products
  2. ISO 14644 – Cleanrooms and associated controlled environments

**Product Label Symbols**

| | |
|---|---|
| REF | Indicates the manufacturer's catalogue number so that the product can be identified. |
| LOT | Indicates the manufacturer's batch code so that the batch or lot can be identified.<br>**NOTE** Synonyms for batch code are lot number and batch number. |
|  | Indicates the date after which the product is not to be used. |
|  | Indicates the temperature limits to which the product can be safely exposed. |
| STERILE A | Indicates a product that has been manufactured using accepted aseptic techniques. |

# 11 Related Products

| Product | Cat. No. |
|---|---|
| Dulbecco's PBS (w/o Ca & Mg) | 02-023-1 |
| MSC NutriStem® XF Medium | 05-200-1 |
| MSC NutriStem® XF Supplement Mix | 05-201-1 |

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The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote all genders.



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