Macrophage Generation Media XF

Instruction Manual

Macrophage Generation Media XF

Product	Size	Catalog Number
M1-Macrophage Generation Medium XF	250 ml	C-28055
M2-Macrophage Generation Medium XF	250 ml	C-28056

Macrophage Base Medium

Product	Size	Catalog Number
Macrophage Base Medium XF	250 ml	C-28057

Monocyte Attachment Medium

Product	Size	Catalog Number
Monocyte Attachment Medium (Ready-to-use)	250 ml	C-28051

Recommended for

- Human Mononuclear Cells (hMNC), freshly isolated
- Human Monocytes (hMo), freshly isolated
- Cryopreserved Human M1 Macrophages (GM-CSF) monocyte-derived
- Cryopreserved Human M2 Macrophages (M-CSF) monocyte-derived

Product Description

Our Macrophage Generation Media have been developed for the efficient generation of monocyte-derived Macrophages (MDM) from freshly isolated peripheral blood monocytes or directly from peripheral blood mononuclear cells (PBMC) as a starting material. In the latter case, the Monocyte Attachment Medium (C-28051) is also needed. The M1- and M2-Macrophage Generation Media XF (C-28055 or C-28056) are ready-to-use media including cytokines for the directed differentiation of M1- or- M2-like polarized MDM. The Macrophage Base Medium XF is the user-customizable version of the Macrophage Generation Media XF and does not include cytokines.

The Macrophage Media XF are serum-free and xeno-free formulations for use with freshly isolated cells and cryopreserved human monocyte-derived macrophages. Due to the utilization of exclusively synthetic, recombinant or plant-sourced materials, human serum albumin, purified from human plasma, is the only non-recombinant protein contained in this medium. Our Macrophage Media XF consist of a bottle of Basal Medium and one vial of SupplementMix. Adding the SupplementMix to the Basal Medium results in the complete Medium. All Macrophage Media XF must be supplemented with additional cytokines. Cytokines are included with the Macrophage Generation Media XF but not with the Macrophage Base Medium XF.

Supplementation Details

PromoCell Macrophage Generation Media XF contain all growth factors and supplements. The Macrophage Base Medium comes without cytokines and must therefore be adequately supplemented by the user. The Monocyte Attachment Medium does not need any further supplementation. Macrophage Generation/Base Media XF and Monocyte Attachment Medium do not contain antibiotics or antimycotics and are formulated for use in an incubator with an atmosphere of 5% CO_2.

Preparation of the Supplemented Medium for Use

Thaw the SupplementMix at 15–25°C. Aseptically mix the supplement solution by carefully pipetting up and down. Then, transfer the entire content of the SupplementMix to the Basal Medium. Close the bottle and swirl gently until a homogenous mixture is

formed. The corresponding Cytokine Mix accompanying the Macrophage Generation Media XF (Cytokine Mix M1 or M2) is delivered as a 100x stock. Immediately before use of the Macrophage Generation Media XF thaw the Cytokine Mix at 15–25°C. Aseptically transfer the appropriate volume of Cytokines and corresponding volume of complete Medium. Close the bottle and swirl gently until a homogenous mixture is formed.

The Macrophage Base Medium XF is supplied without cytokines, which must be added by the user.

Storage and Stability

Store the Basal Medium at 4–8°C in the dark, store the SupplementMix at -20°C immediately after arrival. Keep the Cytokine Mix at -20°C for long-term stor-age or at 4–8°C for up to 2 weeks. Do not freeze the Basal Medium. If stored properly, the products are stable until the expiry date stated on the label. After adding the SupplementMix to the Basal Medium, the shelf life of the complete medium is 6 weeks at 4–8°C. Complete Medium supplemented with cytokines should be used within 2 weeks. Do not freeze the complete medium. For use, prewarm only an aliquot of the complete medium at 15–25°C and keep the remaining medium refrigerated at 4–8°C.

Quality Control

All lots of PromoCell Macrophage Media XF are subjected to comprehensive quality control tests using human peripheral blood mononuclear cells. Each lot of PromoCell Macrophage Media XF is tested for the ability to support macrophage differentiation verified by morphological evaluation and flow cytometry analysis. Each lot of PromoCell Monocyte Attachment Medium is tested for its ability to support optimal attachment and viability of peripheral blood monocytes. Approved in-house lots of media are used as a reference.

In addition, all lots of media have been tested for the absence of microbial contaminants (fungi, bacteria, mycoplasma).

Intended Use

The products are for *in vitro* use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

For detailed information and illustrated step-by-step protocols, please access: www.promocell.com/application-notes

Macrophage Differentiation from freshly isolated PBMC

This protocol describes the *in vitro* differentiation of M1 or M2 macrophages directly from freshly isolated peripheral blood mononuclear cells (PBMC) using the M1- or M2-Macrophage Generation Medium XF, respectively.

Differentiation protocol

Materials

- Monocyte Attachment Medium (C-28051)
- M1- or M2-Macrophage Generation Medium XF (C-28055 or C-28056)
- PBS without Ca^{++}/Mg^{++} (C-40232)
- PBS without Ca^{++}/Mg^{++} (C-40232)/2 mM EDTA/0.1% has
- Optional: Lymphocyte Separation Medium 1077 (C-44010)
- Optional: additional activation/polarization factors (refer to protocol step 8)
- Optional: Macrophage Detachment Solution (C-41330, refer to protocol step 11)

Use aseptic techniques and a laminar flow bench.

1

Isolate Mononuclear Cells (Day 0)

Isolate fresh PBMC from buffy coats using your routine protocol or by using the Lymphocyte Separation Medium 1077. For the separation of vital mononuclear cells with the usage of the Lymphocyte Separation Medium 1077 follow the corresponding instruction manual which is available for download on our website www.promocell.com. Tuesday is a good day to start in order to avoid weekend work.

Note: Do not use buffy coats older than 20 hours, since this will significantly impair the experimental outcome. Buffy coats <8 hours old are optimal.

2

Analyze Mononuclear Cells (Day 0)

Count and analyze the isolated PBMC for monocyte content, (e.g. using the FSC/SSC plot of a flow cytometer). Subsequently resuspend the cells at 100 million PBMC per ml in Monocyte Attachment Medium.

Note: The protocol may be performed without the determination of the monocyte content of the PBMC (see step 3). However, this could result in lower yield due to suboptimal initial plating density.

3

Let the monocytes attach (Day 0)

Plate freshly isolated PBMC in an appropriate amount of Monocyte Attachment Medium, e.g. 15 ml Medium per T-75 flask. Use a seeding density of 1 million/cm² for Mononuclear Cells with a monocyte content of ≥25% and 1.5 million/cm² for a monocyte content of <25%. Incubate for 1–1.5 hours at 5% CO_2 and 37°C in the incubator without any further manipulation.

Note: Use Nunc plasticware with Nunclon™ surface for best results. Use a plating density of 1.5 million PBMC per cm² if step 2 was missed.

4

Prepare the complete Macrophage Generation Medium XF (Day 0)

Prepare the Macrophage Generation Medium XF by adding the thawed SupplementMix aseptically to the Basal Medium. Swirl gently to obtain a homogeneous mixture. Then, add Cytokine Mix M1 or M2, respectively.

5

Wash the adherent cell fraction (Day 0)

Loosen non-adherent cells and aspirate them by vigorously swirling the tissue culture vessel. Wash the adherent cells, i.e. monocytes, three times with warm Monocyte Attachment Medium by swirling the vessel and aspirating the supernatant.

6

Start the macrophage differentiation (Day 0)

Add an appropriate amount of complete M1- or M2-Macrophage Generation Medium XF to the cells, e.g. 20 ml per T-75 flask and incubate for 6 days at 37°C and 5% CO_2 without medium change.

Note: The monocytes differentiate to M1-like or M2-like polarized macrophages under these conditions. If required, activation and subtype-specific polarization can be achieved by performing an optional activation step (refer to step 8).

7

Continue the differentiation process (Day 6)

Add another 50% to 75% by volume of fresh complete M1- or M2-Macrophage Generation Medium XF to the cells. Incubate the immature macrophages for another 3 days at 37°C and 5% CO_2.

Note: Both adherent and suspension cells may be present. Do not remove any used medium, simply add fresh medium.

8

Optional step: macrophage activation (Day 7)

For specific macrophage activation the whole volume of the culture must be supplemented with adequate stimuli of the users´ choice. A medium change is not required, only addition of activation factors. Examples of macrophage activation by defined stimuli include:

- Classically activated M1-macrophages generated via addition of IFN-γ (50 ng/ml) and LPS (10 ng/ml) to M1-macrophages
- M2a-activation of M2-macrophages using 20 ng/ml IL-4
- Supplementation with immune complexes and IL-1β or LPS elicits M2b-activation
- IL-10, TGFβ or glucocorticoids lead to M2c-activation of M2-macrophages
- An alternative M1-activated macrophage can be obtained by the activation of M2-macrophages with IFN-γ and LPS

9

Medium change (Day 9)

Aspirate the medium including cells in suspension and collect it in a centrifugation tube. Immediately, pipet fresh complete Macrophage Generation Medium XF supplemented with appropriate cytokines/ activation factors to the cells. Centrifuge the cells in the tube for 15 min at 350 x g at room temperature. Discard the supernatant and carefully resuspend the cells in a small amount of fresh medium. Combine the resuspended cells in the tube with the adherent cells in the fresh medium contained in the tissue culture vessel. Incubate till the next day at 37°C and 5% CO_2.

Note: Adherent as well as non-adherent cells may be observed at this stage.

10

The macrophages are ready (Day 10)

The macrophages may now be used directly in the plates where they reside, e.g. when performing phagocytosis assays. Alternatively, they can be harvested (see instructions in optional step 11). The culture can be maintained for up to three weeks by performing weekly medium changes with fresh complete Macrophage Generation Medium XF.

Note: Macrophages appear as adherent cells with typical morphology: prominent nucleus with flatly outspread cytoplasm and multiple pseudopodia

11

Optional step: harvesting/sub-cultivation of macrophages (Day

Aspirate and discard the medium. Wash the adherent macrophages twice with endotoxin-free PBS without Ca^{++}/Mg^{++}. Immediately add an appropriate amount of cold (2–8°C) Macrophage Detachment Solution to the cells, e.g. 25 ml per T-75 flask. Seal the tissue culture vessel and incubate cells for 40 min at 2–8°C. If necessary, incubate another 20 min at room temperature to ensure cell release from the culture surface. Firmly tap the tissue culture vessel to facilitate cell detachment. Make sure most of the cells have already detached or are only loosely adherent to the surface of the tissue culture vessel. Only then use a cell scraper to dislodge the remaining macrophages. Collect the harvested macrophages in centrifugation tubes and dilute 1:1 with PBS/2 mM EDTA/0.1% HSA. Centrifuge cells for 15 minutes at 350 x g at room temperature. Apply two washes with PBS/2 mM EDTA/0.1% HSA to the cells and count them. The macrophages are now ready to be used in your experiments. Note: The percentage of attaching cells after re-seeding depends on the overall health status of the macrophages before detachment and the successful performance of the detachment process. Therefore, some degree of variation is unavoidable.

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