

Macrophage Generation Media DXF

PromoCell

Instruction Manual

■ Macrophage Generation Media DXF

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

■ Macrophage Base Medium

Product	Size	Catalog Number
Macrophage Base Medium DXF	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

Product Description

The PromoCell Macrophage Generation Media have been developed for the efficient generation of monocyte-derived Macrophages (MDM) from freshly isolated peripheral blood monocytes or directly from 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 DXF (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 DXF is the

user-customizable version of the Macrophage Generation Media DXF and does not include cytokines.

The Macrophage Media DXF are chemically defined and xeno-free formulations for use with freshly isolated cells. Due to the utilization of exclusively synthetic, recombinant or plant-sourced materials, this medium is free of all animal-derived components and substances of human origin with Human Serum Albumin as the only exception. PromoCell Macrophage Media DXF consist of a bottle of Basal Medium and one vial of SupplementMix. Adding the SupplementMix to the Basal Medium results in the complete Medium. Indeed, all Macrophage Media DXF must be supplemented with additional cytokines. The cytokines are included with the Macrophage Generation Media DXF but not with the Macrophage Base Medium DXF.

Supplementation Details

PromoCell Macrophage Generation Media DXF 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 DXF as well as the Monocyte Attachment Medium do not contain antibiotics or antimycotics and are formulated for use in an incubator with an atmosphere of 5% CO₂.

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

Use aseptic techniques and a laminar flow bench.

Preparation of the Supplemented Medium for Use

Thaw the SupplementMix at 15 to 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 DXF (Cytokine Mix M1 or M2) is delivered as a 100x stock. Immediately before use of the Macrophage Generation Media DXF thaw the Cytokine Mix at 15 to 25°C. Aseptically transfer the referring amount of Cytokines to the appropriate volume of complete Medium. Close the bottle and swirl gently until a homogenous mixture is formed. The Macrophage Base Medium DXF comes without cytokines and must be adequately supplemented by the user.

Macrophage Differentiation

A) Media and solutions

- Monocyte Attachment Medium (C-28051)
- M1- or M2-Macrophage Generation Medium DXF (C-28055 or C-28056)
- endotoxin-free PBS w/o Ca²⁺ / Mg²⁺ (C-40242)
- endotoxin-free PBS w/o Ca²⁺ / Mg²⁺ (C-40242) / 2 mM EDTA/0.1% HSA
- optional: additional activation/polarization factors (refer to protocol step 8)
- optional: Macrophage Detachment Solution DXF (C-41330, refer to protocol step 11)

B) Differentiation Protocol

1. Isolate Mononuclear Cells (day 0)

Isolate fresh PBMC from buffy coats using your routine protocol. 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 not older than 8 hours 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 might 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% CO₂ 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 when step 2 was skipped.

*Preparation
of the Supple-
mented Medi-
um for Use*

*Macrophage
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4. Prepare the complete Macrophage Generation Medium DXF (day 0)

Prepare the Macrophage Generation Medium DXF by adding the thawed Supplement Mix 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)

By vigorously swirling the tissue culture vessel loosen non-adherent cells and aspirate them. 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 DXF to the cells, e.g. 20 ml per T-75 flask and incubate for 6 days at 37°C and 5% CO₂ 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 the 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 DXF to the cells. Incubate the immature macrophages for another 3 days at 37°C and 5% CO₂.

Note: Adherent as well as suspension cells may be present. Do not remove any of the used medium from the cells, just add the fresh medium.

8. Optional step: macrophage activation (day 7)

For specific macrophage activation the whole volume of the culture is supplemented with adequate stimuli of the customers' choice. Do not perform a medium change, just add the activation factors. Examples of macrophage activation by defined stimuli (see also "Related products"):

Classically activated M1-macrophages can be generated by addition of IFN- γ (50 ng/ml) and LPS (10 ng/ml) to M1-macrophages. M2a-activation of M2-macrophages is achieved by 20 ng/ml IL-4. Supplementation with immune complexes and IL-1 β or LPS will elicit M2b-activation, whilst IL-10, TGF β or glucocorticoids lead to M2c-activation of M2-macrophages. An alternative type of M1-activated macrophage can be obtained by the activation of M2-macrophages with IFN- γ and LPS [2].

9. Medium change (day 9)

Aspirate the medium including suspension cells and collect it in a centrifugation tube. Immediately, pipet fresh complete PromoCell Macrophage Generation Medium DXF 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% CO₂.

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

Macrophage Differentiation

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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). Maintenance of the culture for up to three weeks by performing weekly medium changes with fresh complete Macrophage Generation Medium DXF is possible.

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

11. Optional step: Harvesting/Subcultivation of macrophages (day 10+)

Aspirate and discard the medium. Wash the adherent macrophages twice with endotoxin-free PBS w/o Ca^{2+} / Mg^{2+} . Immediately add an appropriate amount of cold (2-8°C) Macrophage Detachment Solution DXF 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 enforce 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 for your experiments.

Note: The percentage of attaching cells after re-seeding depends on the over-all health status of the macrophages before detachment and the successful performance of the detachment process itself. Thus, some degree of variation is unavoidable.

*Macrophage
Differentiation*

Storage and Stability

Store the Basal Medium at 4 to 8°C in the dark, store the SupplementMix at -20°C immediately after arrival. Keep the Cytokine Mix at -20°C for longterm storage or at 4 to 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 to 8°C. Complete Medium supplemented with cytokines should be used within 2 weeks. Do not freeze the complete medium. For use, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4 to 8°C.

Note: The supplements are delivered thawed and can be frozen after arrival without losing any activity. The Cytokine Mix may be aliquoted before freezing.

Quality Control

All lots of PromoCell Macrophage Media DXF are subjected to comprehensive quality control tests using human peripheral blood mononuclear cells. Each lot of PromoCell Macrophage Media DXF 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.

If you require special media modifications, we offer a Custom Media Service starting at 10 bottles per order. Please ask for details.

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