# Dendritic Cell Generation Media

PromoCell

## Instruction Manual

- DC Generation Media

| Product | Size | Catalog Number |
|---|---|---|
| DC Generation Medium (Ready-to-use) | 250 ml | C-28050 |
| DC Generation Medium DXF (Ready-to-use) | 250 ml | C-28052 |

- DC Base Media

| Product | Size | Catalog Number |
|---|---|---|
| DC Base Medium | 250 ml | C-28053 |
| DC Base Medium DXF | 250 ml | C-28054 |

- Monocyte Attachment Medium

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

### Recommended for

- Human Monocytes (hMo), freshly isolated and cryopreserved
- Human Mononuclear Cells (hMNC), freshly isolated

### Product Description

The PromoCell Dendritic Cell (DC) Generation Media have been developed for the efficient generation of myeloid Dendritic Cells, e.g. from peripheral blood monocytes.

PromoCell DC Generation Medium (C-28050) provides optimal culture conditions for the generation of DCs from cryopreserved as well as freshly isolated cells. In the latter case, the Monocyte Attachment Medium (C-28051) is also needed. The DC Generation Medium DXF (C-28052) is a chemically defined and xeno-free formulation 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.

The DC Base Media are the user-customizable versions of the DC Generation Media that do not include cytokines. PromoCell DC Media 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 DC Media must be supplemented with additional cytokines. The cytokines are included with the DC Generation Media but not with the DC Base Media.
For detailed information, please see www.promocell.com/application-notes.

### Supplementation Details

PromoCell DC Generation Media contain all growth factors and supplements. The DC Base Media come without cytokines and must therefore be adequately supplemented by the user. The Monocyte Attachment Medium does not need any further supplementation. DC Generation / Base Media 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% $CO_2$.

*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 Pack accompanying the DC Generation Media contains Component A and B and is delivered as a 100x stock. Immediately before use of the DC Generation Media thaw Component A at 15 to 25°C. Aseptically transfer the appropriate amount of Component A to the appropriate volume of complete Medium. Close the bottle and swirl gently until a homogenous mixture is formed.

**Note:** Do not add Component B to the medium at this point!

The DC Base Media come without cytokines and must be adequately supplemented by the user.

## Generation of moDCs from Freshly Isolated Cells

For Generation of moDCs from freshly isolated peripheral blood Monocytes or Mononuclear Cells, PromoCell recommend the use of the Dendritic Cell Generation Medium DXF (C-28052). Refer to protocol A for details.
Alternatively, the Dendritic Cell Generation Medium (C-28050) in combination with the Monocyte Attachment Medium (C-28051) may be used. See protocol B for detailed procedure.

**Protocol A: Generation of moDCs from freshly isolated cells using DC Generation Medium DXF (C-28052)**

**1. Let the cells attach (day 0)**
Plate freshly isolated cells in an appropriate amount of PromoCell DC Generation Medium DXF w/o cytokines. Use Mononuclear Cells at a density of 2 - 3 million/$cm^2$ and purified Monocytes at 0.5 million/$cm^2$. Incubate for 1 hour at 5% $CO_2$ and 37°C in the incubator.

**2. 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 three times with warm PromoCell DC Generation Medium DXF w/o cytokines by swirling the vessel and aspirating the supernatant.

**3. Start differentiation into immature moDC (day 0)**
Add an appropriate amount of PromoCell DC Generation Medium DXF supplemented with 1x Component A of the Cytokine Pack moDC DXF and incubate for 3 days at 37°C and 5% $CO_2$.

*Preparation of the Supplemented Medium for Use*

*Generation of moDCs from Freshly Isolated Cells*

*Use aseptic techniques and a laminar flow bench.*

**4. Medium change (day 3)**

Perform a medium change on day 3: Aspirate the medium from the cells and collect it in a centrifugation tube. Immediately, pipet fresh PromoCell DC Generation Medium DXF supplemented with 1x Component A of the Cytokine Pack moDC DXF to the cells. Centrifuge the cells in the tube for 10 min at 180 x g. Discard the supernatant and carefully resuspend the cells in a small amount of fresh medium. Combine the resuspended cells in the tube with the cells in the fresh medium contained in the tissue culture vessel. Incubate the immature moDCs for another 3 days at 37°C and 5% $CO_2$.

**Note:** Adherent / loosely attached as well as non-adherent cells may be observed at this stage. Immature moDC, also termed "veiled cells", appear as irregularly outlined cells, occasionally exhibiting large cytoplasmic processes. They show a $CD45^+$ / $CD83^-$ phenotype and stain negative to moderately positive for CD14.

**5. Complete moDC maturation process (day 6)**

To complete the moDC maturation process, supplement the whole volume with 1x of Component B of the Cytokine Pack moDC DXF on day 6. Do not change the medium. Incubate at 37°C and 5% $CO_2$ for an additional 24 - 48 hours.

**6. Harvest mature moDC (day 7 / 8)**

Dislodge loosely attached cells by pipetting up and down several times. Transfer the medium containing the cells to a 50 ml tube. Spin down harvested moDCs at 180 x g for 10 minutes and discard the supernatant.

**Note:** Mature moDCs are non-adherent cells and exhibit a unique morphology originating from their multiple long thread-like dendrites.

**7. Perform your experiments**

Resuspend and count the cells. The moDCs are now ready to be used in your experiments. Optionally, characterize their dendritic cell immunophenotype, e.g. by performing flow cytometry analysis for CD14, CD45 and CD83.

**Note:** Mature moDCs generated in the PromoCell DC Generation Medium DXF supplemented with the Cytokine Pack moDC DXF exhibit a $CD14^-$ / $CD45^+$ / $CD83^+$ phenotype.

*Use aseptic techniques and a laminar flow bench.*

## Generation of moDCs from Freshly Isolated Cells

**Protocol B: Generation of moDCs from freshly isolated cells using DC Generation Medium (C-28050) and Monocyte Attachment Medium (C-28051)**

**1. Let the cells attach (day 0)**

Plate freshly isolated cells in an appropriate amount the PromoCell Monocyte Attachment Medium. Plate Mononuclear Cells at a density of 2 - 3 million/cm$^2$ and purified Monocytes at 0.5 million/cm$^2$. Incubate for 1 hour at 5% $CO_2$ and 37°C in the incubator.

**2. 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 three times with warm Monocyte Attachment Medium by swirling the vessel and aspirating the supernatant.

**3. Start differentiation into immature moDC (day 0)**

Add an appropriate amount of PromoCell Dendritic Cell Generation Medium supplemented with 1x Component A of the Cytokine Pack moDC and incubate for 3 days at 37°C and 5% $CO_2$.

**4. Medium change (day 3)**

Perform a medium change on day 3: Aspirate the medium from the cells and collect it in a centrifugation tube. Immediately, pipet fresh PromoCell DC Generation Medium supplemented with 1x Component A of the Cytokine Pack moDC to the cells. Centrifuge the cells in the tube for 10 min at 180 x g. Discard the supernatant and carefully resuspend the cells in a small amount of fresh medium. Combine the resuspended cells in the tube with the cells in the fresh medium contained in the tissue culture vessel. Incubate the immature moDCs for another 3 days at 37°C and 5% $CO_2$.

**Note:** Adherent / loosely attached as well as non-adherent cells may be observed at this stage. Immature moDC, also termed "veiled cells", appear as irregularly outlined cells, occasionally exhibiting large cytoplasmic processes. They show a CD45$^+$ / CD83$^-$ phenotype and stain negative to moderately positive for CD14.

**5. Complete moDC maturation process (day 6)**

To complete the moDC maturation process, supplement the whole volume with 1x of Component B of the Cytokine Pack moDC on day 6. Do not change the medium. Incubate at 37°C and 5% $CO_2$ for an additional 24 - 48 hours.

**6. Harvest mature moDC (day 7 / 8)**

Dislodge loosely attached cells by pipetting up and down several times. Transfer the medium containing the cells in a 50 ml tube. Spin down harvested moDCs at 180 x g for 10 minutes and discard the supernatant.

**Note:** Mature moDCs are non-adherent cells and exhibit a unique morphology originating from their multiple long thread-like dendrites.

**7. Perform your experiments**

Resuspend and count the cells. The moDCs are now ready to be used in your experiments. Optionally, characterize their dendritic cell immunophenotype, e.g. by performing flow cytometry analysis for CD14, CD45 and CD83.

**Note:** Mature moDCs generated in the PromoCell DC Generation Medium supplemented with the Cytokine Pack moDC exhibit a CD14$^-$ / CD45$^+$ / CD83$^+$ phenotype.

*Use aseptic techniques and a laminar flow bench.*

## Generation of moDCs from Cryopreserved Cells

*Generation of moDCs from Cryopreserved Cells*

For Generation of moDCs from cryopreserved peripheral blood Monocytes, PromoCell recommends the use of the Dendritic Cell Generation Medium (C-28050).

**1. Plate the cells (day 0)**

Thaw cryopreserved monocytes in a water bath according to the Instruction Manual delivered with the cells. After thawing, immediately plate them at 0.5 million/cm$^2$ in an appropriate amount of PromoCell Dendritic Cell Generation Medium supplemented with 1x Component A of the Cytokine Pack moDC. Use at least 9 ml medium per vial of cryopreserved cells. Immediately place them in an incubator for 1 day at 37°C and 5% $CO_2$.

**Note:** It is important to leave the cells untouched for at least 16 hours in order to prevent excessive clumping.

**2. Medium change (day 1)**

Aspirate the medium from the cells and collect it in a centrifugation tube. Immediately pipet fresh PromoCell Dendritic Cell Generation Medium supplemented with 1x Component A of the Cytokine Pack moDC to the cells. Centrifuge the cells in the tube for 10 min at 180 x g. Discard the supernatant and carefully resuspend the cells in a small amount of fresh medium. Combine the resuspended cells with the cells in the fresh medium contained in the tissue culture vessel. Incubate for 3 more days.

**3. Medium change (day 4)**

Perform a medium change as described above. Incubate for a further 2 days at 37°C and 5% $CO_2$.

**Note:** Adherent / loosely attached as well as non-adherent cells may be observed at this stage. Immature moDC, also termed "veiled cells", appear as irregularly outlined cells, occasionally exhibiting large cytoplasmic processes. They show a $CD45^+$ / $CD83^-$ phenotype and stain negative to moderately positive for CD14.

**4. Complete moDC maturation process (day 6)**

To complete the moDC maturation process, supplement the whole volume with 1x of Component B of the Cytokine Pack moDC on day 6. Do not change the medium. Incubate at 37°C and 5% $CO_2$ for an additional 24 - 48 hours.

**5. Harvest mature moDC (day 7 / 8)**

Dislodge loosely attached cells by pipetting up and down several times. Transfer the medium containing the cells to a 50 ml tube. Spin down harvested moDCs at 180 x g for 10 minutes and discard the supernatant.

**Note:** Mature moDCs are non-adherent cells and exhibit a unique morphology originating from their multiple long thread-like dendrites.

**6. Perform your experiments**

Resuspend and count the cells. The moDC are now ready to be used in your experiments. Optionally, characterize their dendritic cell immunophenotype, e.g. by performing flow cytometry analysis for CD14, CD45 and CD83.

**Note:** Mature moDCs generated in the PromoCell DC Generation Medium supplemented with the Cytokine Pack DC exhibit a $CD14^-$ / $CD45^+$ / $CD83^+$ phenotype.

## 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 Pack at -20°C for long-term 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 Pack may be aliquoted before freezing.

## Quality Control

All lots of PromoCell DC Media are subjected to comprehensive quality control tests using human peripheral blood mononuclear cells. Each lot of PromoCell DC Media is tested for the ability to support DC maturation 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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