Dendritic Cell Generation Media

Instruction Manual

DC Generation Media

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

DC Base Media

Product	Size	Catalog Number
DC Base Medium (without cytokines)	250 ml	C-28053
DC Base Medium XF (without cytokines)	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 or cryopreserved
- Human Mononuclear Cells (hMNC), freshly isolated

Product Description

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

Our 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 XF (C-28052) is a serum-free and xeno-free formulation for use with freshly isolated cells. 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.

The DC Base Media are the user-customizable versions of the DC Generation Media that do not include cytokines. Our 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. All DC Media must be supplemented with additional cytokines. Cytokines are included with the DC Generation Media but not with the DC Base Media.

For detailed information, access our website: www.promocell.com/application-notes

Supplementation Details

PromoCell DC Generation Media contain all growth factors and supplements. The DC Base Media is supplied without cytokines, which must be added by the user. The Monocyte Attachment Medium does not need any further supplementation. DC Generation / Base Media 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 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 15-25°C. Aseptically transfer the appropriate volume of Component A to the corresponding

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 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 Pack at -20°C for long-term storage 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, pre-warm only an aliquot of the complete medium and keep the remaining medium refrigerated at 4- 8°C.

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.

Generation of Monocyte-Derived Dendritic Cells (MoDCs)

For generation of moDCs from freshly isolated peripheral blood monocytes or mononuclear cells, we recommend the use of the Dendritic Cell Generation Medium XF (C-28052). Refer to protocol I for details.

Alternatively, the Dendritic Cell Generation Medium (C-28050) in combination with the Monocyte Attachment Medium (C-28051) may be used. See protocol II for detailed procedure.

For generation of moDCs from cryopreserved peripheral blood monocytes, we recommend the use of the Dendritic Cell Generation Medium (C-28050). Please see protocol III for details.

I. Generation of moDCs from freshly isolated cells using DC Generation Medium XF

Materials

- DC Generation Medium XF (C-28052)
- Freshly isolated human mononuclear cells (hMNC) or freshly isolated human monocytes (hMo)

Use aseptic techniques and a laminar flow bench.

1

Let the cells attach (Day 0)

Plate freshly isolated cells in an appropriate amount of DC Generation Medium XF w/o cytokines. Use a seeding density of 2–3 million/cm^2 for mononuclear cells and 0.5 million/cm^2 for purified monocytes. Incubate for 1 hour at 5% CO_2 and 37°C in the incubator.

2

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 three times with warm DC Generation Medium XF w/o cytokines by swirling the vessel and aspirating the supernatant.

3

Start the differentiation into immature moDC (Day 0)

Add an appropriate amount of complete DC Generation Medium XF supplemented with 1x Component A of the Cytokine Pack moDC XF 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 DC Generation Medium XF supplemented with 1x Component A of the Cytokine Pack moDC XF 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 of the culture with 1x of Component B of the Cytokine Pack moDC XF on day 6. A medium change is not required. 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 DC Generation Medium XF supplemented with the Cytokine Pack moDC XF exhibit a $CD14^-$/ $CD45^+$/$CD83^+$ phenotype.

II. Generation of moDCs from freshly isolated cells using DC Generation Medium

Materials

- DC Generation Medium (C-28050)
- Monocyte Attachment Medium (C-28051)
- Freshly isolated Human Mononuclear Cells (hMNC) or freshly isolated Human Monocytes (hMo)

Use aseptic techniques and a laminar flow bench.

1

Let the cells attach (Day 0)

Plate freshly isolated cells in an appropriate amount of Monocyte Attachment Medium. Use a seeding density of 2–3 million/cm² for mononuclear cells and 0.5 million/cm² for purified monocytes. Incubate for 1 hour at 5% CO_2 and 37°C in the incubator.

2

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

3

Start the differentiation into immature moDC (Day 0)

Add an appropriate amount of complete 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 complete 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 of the culture 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 complete DC Generation Medium supplemented with the Cytokine Pack moDC exhibit a $CD14^-/CD45^+/CD83^+$ phenotype.

III. Generation of moDCs from cryopreserved cells using DC Generation Medium

Materials

- DC Generation Medium (C-28050)
- Human CD14+ Monocytes (hMoCD14+-PB, C-12909)

Use aseptic techniques and a laminar flow bench.

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 complete 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 complete Dendritic Cell Generation Medium supplemented with 1x Component A of the Cytokine Pack moDC to the cells. Centrifuge the cells 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.

If you require special media modifications, we offer a Custom Media Service starting at 10 bottles per order.
Contact us at info@promocell.com to find out more.

PromoCell GmbH
Sickingenstr. 63/65
69126 Heidelberg
Germany

info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 96 03 33 (toll free)
Fax: 0800 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40

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