

Application Note

Background

Dendritic Cells (DCs) are so called because of their characteristic cell surface projections that resemble the dendrites of neurons (see Fig. 1 and 3). These highly motile immune cells are found virtually everywhere in the body and represent a heterogeneous group of cells sharing the same function: dendritic cells are the most powerful antigen presenting cells (APCs) of the mammalian immune system [1]. Being responsible for the induction of T or T-dependent immunity and tolerance, they are especially abundant in epithelia, e.g. skin and intestinal tract, the ideal location to encounter invading pathogens [2]. DCs continuously sample their environment for antigens by means of endocytosis [1].

Researchers hold great promise for dendritic cells in the development of cancer immunotherapies [3] as well as the treatment of autoimmune diseases [4]. Since DCs also play an essential role in the setting of HIV infection [5] and the pathogenesis of several other viruses, they have significance as a therapeutical target. Currently, there are two known major subsets of dendritic cells in humans: firstly lymphoid dendritic cells derive from plasmacytoid cells in blood, and myeloid dendritic cells, the second group, arise from myeloid precursor cells, e.g. peripheral blood monocytes or CD34⁺ progenitors from bone marrow [1]. DCs develop by means of a maturation process induced by exogenous or endogenous stimuli (see Fig. 1). PromoCell's Dendritic Cell Generation

Media allow for the efficient and reliable *in vitro* maturation of human Monocytes (hMo) into immature as well as fully mature CD83⁺ monocyte-derived Dendritic Cells (moDCs, see Fig. 5). The chemically defined and xeno-free PromoCell Dendritic Cell Generation Medium DXF (C-28052) is recommended for use with freshly isolated Monocytes or Mononuclear Cells (MNCs). For the differentiation of cryopreserved purified Monocytes into moDCs, use the PromoCell Dendritic Cell Generation Medium (C-28050). The latter one may also be used with freshly isolated Monocytes and MNCs when combined with the PromoCell Monocyte Attachment Medium (C-28051). Please, refer to the step-by-step protocols below for details.

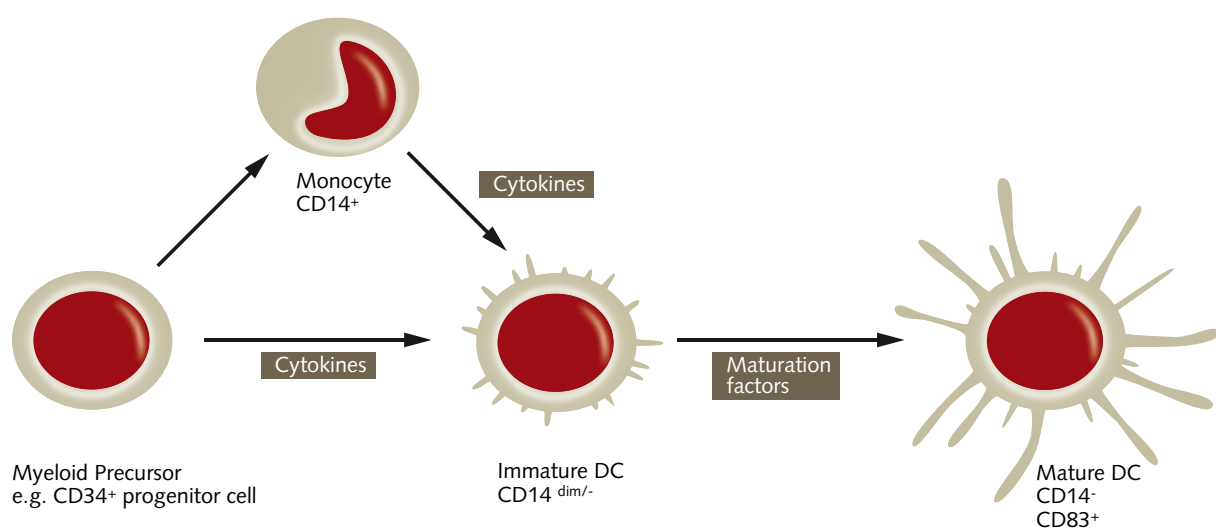


Fig. 1: Maturation scheme of myeloid Dendritic Cells

Use aseptic techniques and a laminar flow bench.

Generation of moDCs from Freshly Isolated Cells

For Generation of moDCs from freshly isolated peripheral blood Monocytes or Mononuclear Cells, PromoCell recommends 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. Mononuclear Cells at a density of 2-3 million/cm² and purified Monocytes at 0.5 million/cm². Incubate for 1 hour at 5% CO₂ 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 (supplied at 100x) and incubate for 3 days at 37°C and 5% CO₂.

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% CO₂.

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 ("veils", see Fig. 2). 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 (supplied at 100x) on day 6. Do not change the medium. Incubate at 37°C and 5% CO₂ for an additional 24-48 hours.

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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 (see Fig. 3).

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 immuno-phenotype, 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 exhibit a CD14⁻ / CD45⁺ / CD83⁺ phenotype (see Fig. 4 and 5).

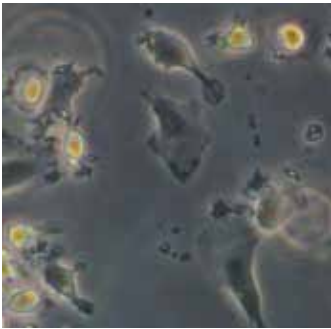


Fig. 2: Day 6 immature monocyte-derived Dendritic Cells (moDCs). Note the large cytoplasmic, veil-like processes seen in adherent (center) as well as loosely attached (upper left) cells.

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 of the PromoCell Monocyte Attachment Medium. Plate Mononuclear Cells at a density of 2-3 million/cm² and purified Monocytes at 0.5 million/cm². Incubate for 1 hour at 5% CO₂ 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 (supplied at 100x) and incubate for 3 days at 37°C and 5% CO₂.

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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% CO₂.

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 (“veils”, see Fig. 2). 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 (supplied at 100x) on day 6. Do not change the medium. Incubate at 37°C and 5% CO₂ 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 (see Fig. 3).

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 immuno-phenotype, 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 (see Fig. 4 and 5).

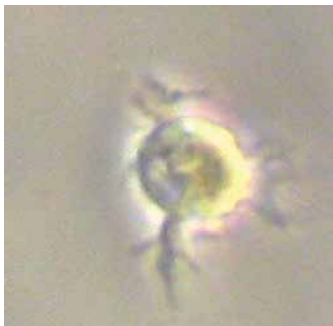


Fig. 3: Day 8 mature monocyte-derived Dendritic Cell (moDC). Note the multiple dendrite-like structures arising from the surface of this non-adherent cell.

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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 waterbath according to the Instruction Manual delivered with the cells. After thawing, immediately plate them at 0.5 million/cm² in an appropriate amount of PromoCell Dendritic Cell Generation Medium supplemented with 1x Component A of the Cytokine Pack moDC (supplied at 100x). 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% CO₂.

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% CO₂.

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 ("veils", see Fig. 2). 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 (supplied at 100x) on day 6. Do not change the medium. Incubate at 37°C and 5% CO₂ for an additional 24 to 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.

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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 immuno-phenotype, 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 (see Fig. 4 and 5).

Generation of moDCs from Cryopreserved Cells

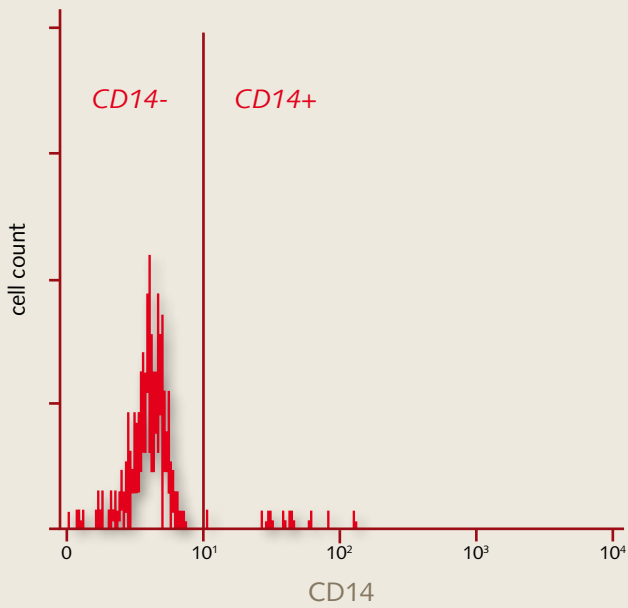


Fig. 4: Flow-cytometry analysis of day 8 mature moDCs generated in the PromoCell DC Generation Medium. More than 98% of the cells are negative for CD14.*

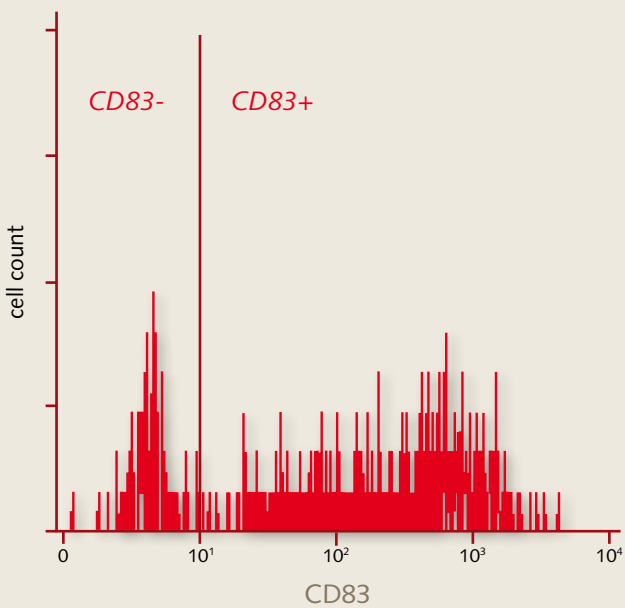


Fig. 5: Flow-cytometry analysis of day 8 mature moDCs generated in the PromoCell DC Generation Medium. More than 76% of the cells are positive for the DC maturation marker CD83.*

* Best results are observed with fresh cells from peripheral blood / buffy coats isolated within 8 hours of blood collection. Note that the yield of *in vitro* generated moDC may vary based on donor variation and the cell source used. Expect cell loss (monocytes) within the first 2 days of the DC Generation procedure.



References

- [1] Chung NP, Chen Y, Chan VS, Tam PK, Lin CL (2004). Dendritic cells: sentinels against pathogens. *Histol Histopathol*, 19(1):317-24.
- [2] Kubach J, Becker C, Schmitt E, Steinbrink K, Huter E, Tuetttenberg A, Jonuleit H (2005). Dendritic cells: sentinels of immunity and tolerance. *Int J Hematol*, 81(3):197-203.
- [3] Banchereau J, Palucka AK (2005). Dendritic cells as therapeutic vaccines against cancer. *Nat Rev Immunol*, 5(4):296-306.
- [4] Ohnmacht C, Pullner A, King SBS, Drexler I, Meier S, Brocker T, Voehringer D (2009). Constitutive ablation of dendritic cells breaks self-tolerance of CD4 T cells and results in spontaneous fatal autoimmunity. *J Exp Med*, 206(3):549–59.
- [5] Lo P (2003). HIV hijacks dendritic cells. *Nat Med*, 9:650.

Related Products

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 Medium	250 ml	C-28053
DC Base Medium DXF	250 ml	C-28054
Monocyte Attachment Medium (Ready-to-use)	250 ml	C-28051
Human CD34 ⁺ Progenitor Cells from Cord Blood (hCD34 ⁺ -CB), single donor	100,000 cryopreserved cells	C-12921
Mononuclear Cell Medium (Ready-to-use)	500 ml	C-28030
IL-6, human, recombinant	20 µg	C-61625
GM-CSF, human, recombinant (E. coli)	10 µg	C-60420
IL-1 beta, human, recombinant	10 µg	C-61120
IL-4 CC, human, recombinant	5 µg	C-61401
sCD40 Ligand (CD154), human, recombinant	50 µg	C-63100
TNF-alpha, human, recombinant (E. coli)	50 µg	C-63719
IFN-alpha 1, human, recombinant (E. coli)	20 µg	C-60577

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: 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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