OptiPrep™ Application Sheet C46

Isolation of dendritic cells from tissues by sedimentation on to a density barrier

- OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- The **OptiPrep™ Mini-Review "Dendritic cells from blood and tissues" (MC06)** compares all of the currently available methodologies and provides a comprehensive bibliography of all the published papers reporting the use of OptiPrep™ listed according to the research topic: to access return to the initial list of Folders and select "**Mini-Reviews**"
- **Section 6** of this Application Sheets lists the published papers reporting the use of the barrier sedimentation technique listed according to tissue source
- To access other Application Sheets referred to in the text return to the Cell Index; key Ctrl "F" and type the C-Number in the Find Box

1. Background

Since dendritic cells (DC) were recognized as playing an important role in the induction of cell-mediated responses [1], there has been a rapid growth in research into the function of these cells and methods for their purification. Gradients of either albumin or metrizamide, although providing an effective enrichment of DC, tended to cause some functional alteration of the cells (see ref 2 for details). However, because cells are more tolerant of Nycodenz®, this iodinated density gradient medium rapidly became established as the medium of choice for DC cell purification from peripheral blood and from lymphoid tissues.

Nycodenz® density barriers of ρ = 1.076 g/ml [3] to 1.084 g/ml [4], the majority being approx 1.077 g/ml (e.g. refs. 5-9), have been used in the "traditional" format in which the crude cell fraction is layered on top. There are also instances of the use of the customized medium Nycoprep™ 1.068 [10,11], which was designed for the purification of monocytes from a human leukocyte-rich plasma. This strategy of layering the cell suspension on top of a barrier has been extended to the use of OptiPrep™ for isolation of DCs from lymph [12], bone marrow [13], bone marrow cell cultures [14], spleen [15], thymus [16] and lung [17].

- For alternative flotation protocols see **Application Sheet C20 and Application Sheet C21**

2. Preparation of cells

For the standard preparation of DCs from spleen and thymus by collagenase digestion and filtration see **Application Sheet C21**. Lung DCs are prepared by perfusion of the tissue via the pulmonary artery with phosphate-buffered saline containing heparin prior to digestion with collagenase (see ref. 15 for more details). For the preparation of DCs from bone marrow cell cultures, the cells flushed from murine femurs and tibias are cultured in the standard RPMI/10% FCS in the presence of 10 ng/ml IL-4 and granulocyte-macrophage colony-stimulating factor for 6 days prior to the harvesting of loosely adherent and non-adherent cells (see ref. 14 or more details of this cell preparation protocol).

3. Purification of dendritic cells (adapted from ref 14)

3a. Solutions required

A. OptiPrep™ (shake the bottle gently before use)

B. Diluent: 0.88% (w/v) NaCl, 1 mM EDTA, 0.5% bovine serum albumin (BSA), 10 mM Tricine-NaOH, pH 7.4 (see Note 1)

C. Density barrier: 2.3 vol. of Solution A + 9.7 vol. of Solution B (see Note 2)

D. Hank's Balanced Salt Solution (see Note 3)

Keep the following stock solutions at 4°C:
100 mM Tricine: 1.79 g per 100 ml water
100 mM EDTA ($Na_2.2H_2O$): 3.72g per 100 ml water

Solution B: Dissolve 0.88 g NaCl in 50 ml water; add 10 ml and 1 ml respectively of stock buffer and EDTA solutions; adjust to pH 7.4 with NaOH; dissolve 0.5 g of bovine serum albumin in the solution; check the pH and make up to 100 ml.

3b. Protocol

Carry out all operations at 4°C, making sure that all solutions and equipment are pre-cooled.

1. Harvest the cells by centrifugation at 540 *g* for 5 min and wash them twice in Solution D.

2. Suspend the washed cell pellet in this solution (6 ml).

3. Transfer 6 ml of Solution C or other chosen density barrier to a centrifuge tube and overlay with cell suspension (see Note 4).

4. Centrifuge at 600-700 *g* for 5-10 min; use a slow acceleration if available (see Note 5).

5. Allow the rotor to decelerate without the brake and harvest the DCs from the interface (see Note 6).

4. Notes

1. In some cases the OptiPrep diluent is a culture medium supplemented with FCS and EDTA. Any approximately isoosmotic solution compatible with the cells may be used.
2. The density of this barrier is approx. 1.067 g/ml and equivalent to 11.6% (w/v) iodixanol; others have used 12% [15]. For lung DCs Hansen et al [17] employed a two-layer gradient of 4% and 16% (w/v) iodixanol.
3. The solution used to suspend the cells prior to layering on the density barrier and the solution used to dilute the OptiPrep™ may be any compatible isoosmotic medium. Hansen et al [17] used Hank's Balanced Salt Solution (HBSS) containing 5% FCS and 2 mM EDTA for both, while Cervantes-Barragan et al [15] replaced the HBSS with PBS and Luckashenak et al [16] used PBS for suspending the cells and HBSS for diluting the OptiPrep™ (both containing 0.2% BSA and 2 mM EDTA.
4. The relative volumes of sample and density barrier are probably not very critical; 2ml over 2 ml is a quite common format with Nycodenz® barriers.
5. The time may be increased to 20 min. Most centrifugations are carried out at 4°C but higher temperatures may be used. There is a broad range of centrifugation conditions with Nycodenz® barriers; approx. 800 *g* for 20 min is common but 530 *g* for 20 min to 2800 *g* for 15 min have been described.
6. In the case of the two-layer gradient, the DCs band at the lower interface. Eaton et al [14] reported that the purity of the DCs from bone marrow cultures, as judged by flow cytometry, was approx. 85%. The harvested DC may be further purified by negative selection using the appropriate MAb-coated magnetic beads. Carrying out this initial gradient purification step allows the bead purification by negative selection to be performed more efficiently. Ref. 19 reviews DC methodology and function.

5. References

1. Barfoot, R., Denham, S., Gyure, L. A., Hall, J. G., Hobbs, Jackson, L. E. and Robertson, D. (1989) *Some properties of dendritic macrophages from peripheral lymph* Immunology, **68**, 233-239
2. McLellan, A. D., Starling, G. C. and Hart, D. N. J. (1995) *Isolation of human blood dendritic cells by discontinuous Nycodenz gradient centrifugation* J. Immunol. Meth., **184**, 81-89
3. Durant, S., Alves, V., Coulaud, J. and Homo-Delarche, F. (2002) *Nonobese diabetic (NOD) mouse dendritic cells stimulate insulin secretion by prediabetic islets* Autoimmunity, **35**, 449-4551059
4. Jang, M.H., Sougawa, N., Tanaka, T., Hirata, T., Hiroi, T., Tohya, K., Guo, Z., Umemoto, E., Ebisuno, Y., Yang, B-G., Seoh, J-Y., Lipp, M., Kiyono, H. and Miyasaka, M. (2006) *CCR7 is critically important for migration of dendritic cells in intestinal lamina propria to mesenteric lymph nodes* J. Immunol., **176**, 803-810
5. Simons, P.J., Delemarre, F.G.A. and Drexhage, H.A. (1998) *Antigen-presenting dendritic cells are regulators of the growth of thyrocytes: a role of interleukin-1β and interleukin-6* Endocrinology, **139**, 3148-3156
6. Leenen, P.J.M, Radosevic, K., Voerman, J.S.A., Salomon, B., van Rooijen, N., Klatzmann, D. and van Ewijk, W. (1998) *Heterogeneity of mouse spleen dendritic cells: in vivo phagocytic activity, expression of macrophage markers, and subpopulation turnover* J. Immunol., **160**, 2166-2173

7. Den Haan, J.M.M., Lehar, S.M. and Bevan, M.J. (2000) *$CD8^+$ but not $CD8^-$ cells cross-prime cytotoxic T cells in vivo* J. Exp. Med., **192**, 1685-1695
8. Roberts, J.M., Yang, J. and Ronchese, F. (2004) *IL-4 deficiency does not impair the ability of dendritic cells to initiate $CD4^+$ and $CD8^+$ T cell responses in vivo* Int. Immunol., **16**, 1451-1458
9. Trinite, B., Chauvin, C., Pêche, H., Voisine, C., Heslan, M. and Josien, R. (2005) *Immature $CD4^-CD103^+$ rat dendritic cells induce rapid caspase-independnet apoptosis-like cell death in various tumor and nontumor cells and phagacytose their victims* J. Immunol., **175**, 2408-2417
10. Moghaddami, M., Swart, B., Reynolds, P., Diener, K. and Brown, M.P. (2002) *Flt3 ligand expands dendritic cell numbers in normal and malignant murine prostate* Immunol. Cell Biol., **80**, 370-381
11. Chen, L., Arora, M., Yarlagadda, M., Oriss, T.B., Krishnamoorthy, N., Ray, A. and Ray, P. (2006) *Distinct responses of lung and spleen dendritic cells to the TLR9 agonist CpG oligodeoxynucleotide* J. Immunol., **177**, 2373-2383
12. Bonneau, M., Epardaud, M., Payot, F., Niborski, V., Thoulouze, M-I., Bernex, F., Charley, B., Riffault, S., Guilloteau, L.A. and Schwartz-Cornil, I. (2006) *Migratory monocytes and granulocytes are major lymphatic carriers of Salmonella from tissue to draining lymph node* J. Leukoc. Biol., **79**, 268-276
13. Lee, J-E., Kang, C-S., Guan, X-Y., Kim, B-T., Kim, S-H., Lee, Y-M., Moon, W-S. and Kim, D-K. (2007) *Discoidin domain receptor 2 is involved in the activation of bone marrow-derived dendritic cells caused by type I collagen* Biochem. Biophys. Res. Comm., **352**, 244-250
14. Eaton, K.A., Benson, L.H., Haeger, J. and Gray, B.M. (2006) *Role of transcription factor T-bet expression by CD4+ cells in gastritis due to Helicobacter pylori in mice* Infect. Immun., **74**, 4673-4684
15. Cervantes-Barragan, L., Züst, R., Weber, F., Spiegel, M., Lang, K.S., Akira, S., Thiel, V. and Ludewig, B. (2007) *Control of coronavirus infection through plasmacytoid dendritic cell-derived type I interferon* Blood, **109**, 1131-1137
16. Luckashenak, N.A., Ryszkiewicz, R.L., Ramsey, K.D. and Clements, J.L. (2006) *The Src homology 2 domain-containing leukocyte protein of 76-kDa adaptor links integrin ligation with p44/42 MAPK phosphorylation and podosome distribution in murine dendritic cells* J. Immunol., **177**, 5177-5185
17. Hansen, S., Lo, B., Evans, K., Neophytou, P., Holmskov, U. and Wright, J.R. (2007) *Surfactant protein D augments bacterial association but attenuates major histocompatibility complex class II presentation of bacterial antigens* Am. J. Respir. Cell. Mol. Biol., **36**, 94-102
18. Kronin, V., Winkel, K., Suss, G. Kelso, A., Heath, W., Kirberg, J., von Boehmer, H. and Shortman. K. (1996) *A subclass of dendritic cells regulates the response of naïve CD8 T cells by limiting their IL-2 production* J. Immunol., **157**, 3819-3627
19. Ardavin, C., Martinez del Hoyo, G., Martin, P., Anjuere, F., Arias, C. F., Marin, A. R., Ruiz, S., Parrillas, V. and Hernandez, H. (2001) *Origin and differentiation of dendritic cells* Trends Immunol., **22**, 691-700

6. Tissue source of dendritic cells using the C46 technique

Bone marrow

1. Eaton, K.A., Benson, L.H., Haeger, J. and Gray, B.M. (2006) *Role of transcription factor T-bet expression by CD4+ cells in gastritis due to Helicobacter pylori in mice* Infect. Immun., **74**, 4673-4684
2. Lee, J-E., Kang, C-S., Guan, X-Y., Kim, B-T., Kim, S-H., Lee, Y-M., Moon, W-S. and Kim, D-K. (2007) *Discoidin domain receptor 2 is involved in the activation of bone marrow-derived dendritic cells caused by type I collagen* Biochem. Biophys. Res. Commun., **352**, 244-250
3. Zhang, M., Berndt, B.E., Chen, J-J. and and Kao, J.Y. (2008) *Expression of a soluble TGF-β receptor by tumor cells enhances dendritic cell/tumor fusion vaccine efficacy* J. Immunol., **181**, 3690-3697
4. Williams, M.A., Bauer, S., Lu, W., Guo, J., Walter, S., Bushnell, T.P., Lillehoj, E.P. and Georas, S.N. (2010) *Deletion of the mucin-like molecule Muc1 enhances dendritic cell activation in response to toll-like receptor ligands* J. Innate Immun., **2**, 123–143
5. Pulecio, J., Petrovic, J., Prete, F., Chiaruttini, G., Lennon-Dumenil, A-M., Desdouets, C., Gasman, S. Burrone, O.R. and Benvenuti, F. (2010) *Cdc42-mediated MTOC polarization in dendritic cells controls targeted delivery of cytokines at the immune synapse* J. Exp. Med., **207**, 2719-2732
6. Kodumudi, K.N., Weber, A., Sarnaik, A.A. and Pilon-Thomas, S. (2012) *Blockade of myeloid-derived suppressor cells after induction of lymphopenia improves adoptive T cell therapy in a murine model of melanoma* J. Immunol., **189**, 5147–5154
7. Komine, H., Kuhn, L., Matsushita, N., Mulé, J.J. and Pilon-Thomas, S. (2013) *Examination of MARCO activity on dendritic cell phenotype and function using a gene knockout mouse* PLoS One, **8**: e67795
8. Ghansah, T., Vohra, N., Kinney, K., Weber, A., Kodumudi, K., Springett, G., Sarnaik, A.A. and Pilon-Thomas, S. (2013) *Dendritic cell immunotherapy combined with gemcitabine chemotherapy enhances survival in a murine model of pancreatic carcinoma* Cancer Immunol. Immunother., **62**, 1083–1091
9. Perez-Shibayama, C., Gil-Cruz, C., Nussbacher, M., Allgäuer, E., Cervantes-Barragan, L., Züst, R. and Ludewig, R. (2013) *Dendritic cell-specific delivery of Flt3L by coronavirus vectors secures induction of therapeutic antitumor immunity* PLoS One, **8**: e81442

Langerhans cells

1. Lalor, P.F., Curbishley, S.M. and Adams, D.H. (2010) *Identifying homing interactions in T-cell traffic in human disease* In, T-Cell Trafficking (eds. Marelli-Berg, F.M. and Nourshargh, S.), Methods Mol. Biol., **616**, Springer Science+Business Media, pp. 231-252

Liver

1. Wiegard, C., Wolint, P., Frenzel, C., Cheruti, U., Schmitt, E., Oxenius, A., Lohse, A.W. and Herkel, J. (2007) *Defective T helper response of hepatocyte-stimulated CD4 T cells impairs antiviral CD8 response and viral clearance* Gastroenterology **133**, 2010-2018
2. Lalor, P.F., Curbishley, S.M. and Adams, D.H. (2010) *Identifying homing interactions in T-cell traffic in human disease* In, T-Cell Trafficking (eds. Marelli-Berg, F.M. and Nourshargh, S.), Methods Mol. Biol., **616**, Springer Science+Business Media, pp. 231-252
3. Connolly, M.K., Ayo, D., Malhotra, A., Hackman, M., Bedrosian, A.S., Ibrahim, J., Cieza-Rubio, N.E., Nguyen, A.H., Henning, J.R., Dorvil-Castro, M., Pachter, H.L. and Miller, G. (2011) *Dendritic cell depletion exacerbates acetaminophen hepatotoxicity* Hepatology, **54**, 959-968
4. Cavassani, K.A., Moreira, A.P., Habiel, D., Ito, T., Coelho, A.L., Allen, R.M., Hu, B., Raphelson, J., Carson IV, W.F., Schaller, M.A., Lukacs, N.W., Omary, B., Hogaboam, C.M. and Kunkel, S.L. (2013) *Toll like receptor 3 plays a critical role in the progression and severity of acetaminophen-induced hepatotoxicity* PLoS One, **8**: e65899

Lymph

1. Muixı, L., Contreras, V., Collado, J.A., Alexandre, Y., Ballingall, K., Bonneau, M., Jaraquemada, D. and Schwartz-Cornil, I. (2012) *Unraveling features of the natural MHC class II peptidome of skin-migrated dendritic cells* Int. Immunol., **24**, 59–69
2. Contreras, V., Urien, C., Jouneau, L., Bourge, M., Bouet-Cararo, C., Bonneau, M., Zientara, S., Klonjkowski, B. and Schwartz-Cornil I. (2012) *Canine recombinant adenovirus vector induces an immunogenicity-related gene expression profile in skin-migrated CD11b$^+$ -type DCs* PLoS One, **7**: e52513

Lymph node

1. Bonneau, M., Epardaud, M., Payot, F., Niborski, V., Thoulouze, M-I., Bernex, F., Charley, B., Riffault, S., Guilloteau, L.A. and Schwartz-Cornil, I. (2006) *Migratory monocytes and granulocytes are major lymphatic carriers of Salmonella from tissue to draining lymph node* J. Leukoc. Biol., **79**, 268-276
2. Lalor, P.F., Curbishley, S.M. and Adams, D.H. (2010) *Identifying homing interactions in T-cell traffic in human disease* In, T-Cell Trafficking (eds. Marelli-Berg, F.M. and Nourshargh, S.), Methods Mol. Biol., **616**, Springer Science+Business Media, pp. 231-252

Lung

1. Hansen, S., Lo, B., Evans, K., Neophytou, P., Holmskov, U. and Wright, J.R. (2007) *Surfactant protein D augments bacterial association but attenuates major histocompatibility complex class II presentation of bacterial antigens* Am. J. Respir. Cell. Mol. Biol., **36**, 94-102
2. Ledford, J.G., Lo, B., Kislan, M.M., Thomas, J.M., Evans, K., Cain, D.W., Kraft, M., Williams, K.L. and Wright, J.R. (2010) *Surfactant protein-A inhibits mycoplasma-induced dendritic cell maturation through regulation of HMGB-1 cytokine activity* J. Immunol., **185**, 3884-3894
3. Pastva, A.M., Mukherjee, S., Giamberardino, C., Hsia, B., Lo, B., Sempowski, G.D. and Wright, J.R. (2011) *Lung effector memory and activated CD4$^+$ T cells display enhanced proliferation in surfactant protein A-deficient mice during allergen-mediated inflammation* J. Immunol., **186**, 2842–2849
4. Van de Laar, L., Guilliams, M. and Tavernier, S. (2016) *Isolation of conventional dendritic cells from mouse lungs* In Methods Mol. Biol., **1423**, Dendritic Cell Protocols (eds. Segura, E. and Onai, N.) Springer Science+Business Media LLC, pp 139-152

Mononuclear cells

1. Fiebach, A.R., Guzylack-Piriou, L., Python, S., Summerfield, A. and Ruggli, N. (2011) *Classical swine fever virus Npro limits type I interferon induction in plasmacytoid dendritic cells by interacting with interferon regulatory factor 7* J. Virol., **85**, 8002-8011

Spleen

2. Luckashenak, N.A., Ryszkiewicz, R.L., Ramsey, K.D. and Clements, J.L. (2006) *The Src homology 2 domain-containing leukocyte protein of 76-kDa adaptor links integrin ligation with p44/42 MAPK phosphorylation and podosome distribution in murine dendritic cells* J. Immunol., **177**, 5177-5185
3. Cervantes-Barragan, L., Züst, R., Weber, F., Spiegel, M., Lang, K.S., Akira, S., Thiel, V. and Ludewig, B. (2007) *Control of coronavirus infection through plasmacytoid dendritic cell-derived type I interferon* Blood, **109**, 1131-1137
4. Wiegard, C., Wolint, P., Frenzel, C., Cheruti, U., Schmitt, E., Oxenius, A., Lohse, A.W. and Herkel, J. (2007) *Defective T helper response of hepatocyte-stimulated CD4 T cells impairs antiviral CD8 response and viral clearance* Gastroenterology **133**, 2010-2018
5. Züst, R., Cervantes-Barragán, L., Kuri, T., Blakqori, G., Weber, F., Ludewig, B. and Thiel, V. (2007) *Coronavirus non-structural protein 1 is a major pathogenicity factor: implications for the rational design of coronavirus vaccines* PLoS Pathog., **3**, 1062-1072
6. de Brito, C., Tomkowiak, M., Ghittoni, R., Caux, C., Leverrier, Y. and Marvel, J. (2011) *CpG promotes cross-presentation of dead cell-associated antigens by pre-CD8a+ dendritic cells* J. Immunol., **186**, 1503–1511

7. Nierkens, S., den Brok, M.H., Garcia, Z., Togher, S., Wagenaars, J., Wassink, M., Boon, L., Ruers, T.J., Figdor, C.G., Schoenberger, S.P., Adema, G.J. and Janssen, E.M. (2011) *Immune adjuvant efficacy of CpG oligonucleotide in cancer treatment is founded specifically upon TLR9 function in plasmacytoid dendritic cells* Cancer Res., **71**, 6428–37
8. Perez-Shibayama, C., Gil-Cruz, C., Nussbacher, M., Allgäuer, E., Cervantes-Barragan, L., Züst, R. and Ludewig, R. (2013) *Dendritic cell-specific delivery of Flt3L by coronavirus vectors secures induction of therapeutic antitumor immunity* PLoS One, **8**: e81442
9. Markey, K.A., Gartlan, K.H., Kuns, R.D., MacDonald, K.P.A. and Hill, G.R. (2015) *Imaging the immunological synapse between dendritic cells and T cells* J. Immunol. Meth., **423**, 40–44

Thymus

1. Luckashenak, N.A., Ryszkiewicz, R.L., Ramsey, K.D. and Clements, J.L. (2006) *The Src homology 2 domain-containing leukocyte protein of 76-kDa adaptor links integrin ligation with p44/42 MAPK phosphorylation and podosome distribution in murine dendritic cells* J. Immunol., **177**, 5177-5185

Virus-infected cells

1. Bechelli, J., Smalley, C., Zhao, X., Judy, B., Valdes, P., Walker, D.H. and Fang, R. (2016) *MyD88 mediates instructive signaling in dendritic cells and protective inflammatory response during rickettsial infection* Infect. Immun., **84**, 883-89

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