

OptiPrep™ Application Sheet C41

Purification of malarial parasites (*Plasmodium falciparum*, *Plasmodium berghei*, *Plasmodium vivax*, and *Plasmodium yoelii*)

- ◆ OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- ◆ 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
- ◆ See Section 4 regarding use of OptiPrep™ for these separations

1. Background

The use of a 12.5% (w/v) Nycodenz® density barrier to enrich for either gametocytes or ookinetes from cultures of *Plasmodium falciparum* was first described by Carter et al [1] who reported that viability of the parasites purified in this manner is greater than those purified in Percoll®. Later, a 10% (w/v) Nycodenz® cushion was used to harvest macrogametes and zygotes from *Plasmodium berghei* while if 12% (w/v) Nycodenz® was used the material contained, in addition, ookinetes [2]. In a three-layer gradient of 6%, 11% and 16% (w/v) Nycodenz®, macrogametes and zygotes from *Plasmodium falciparum* banded at the 6%/11% interface [3,4]. This three-layer gradient is a widely used approach for separating various forms of the organism [5-7].

Mons et al [8], who used either a 16% or 16.5% (w/v) Nycodenz® cushion to concentrate the parasites from *Plasmodium vivax* cultures, reported that although the interfacial material contained mainly parasitized erythrocytes, some leukocytes, large erythrocytes and erythrocyte ghosts were observed. The enrichment on the 16% Nycodenz® was noticeably higher (400-4200x) than on the 15% Nycodenz® (10-200x). The 16.5% Nycodenz® barrier was also found to provide an approx. five-fold enrichment of reticulocytes. A barrier of 12-16% (w/v) is widely used to purify a variety of parasitized erythrocytes.

Important Note: in many instances the concentration of Nycodenz® is reported as 50% or 60%; these figures are actually the volume percentage of Nycoprep™ 1.15, which is no longer available commercially. Nycoprep 1.15 was an isoosmotic solution containing 27.6% (w/v) Nycodenz®, thus a 50% (v/v) solution is equivalent to 13.8% (w/v) Nycodenz® and a 60% solution is equivalent to approx 16.5% (w/v) Nycodenz®. Only this concentration format is given in this Application Sheet.

2. Solution preparation

Nycodenz® solutions must now be prepared by dissolution of Nycodenz® powder in a suitable medium. We suggest making a 30% (w/v) Nycodenz® stock solution. To 50 ml of water (stirred gently at 60°C) slowly add 30 g of Nycodenz® until completely dissolved. Allow the solution to cool to room temperature; add 10 ml of 100 mM Tris, HEPES or Tricine; adjust the pH to 7.0-7.5 and make up to 100 ml with water. This stock solution may be filter-sterilized if required for storage. When diluted with a balanced salt solution, buffered saline solution or culture medium, to produce solutions of lower density, these solutions will be approx. isoosmotic with mammalian plasma.

An exception to this preparation of Nycodenz® density gradient solutions from powdered Nycodenz®, is the purification of *Plasmodium falciparum* gametocytes by banding at the interface of a Nycoprep™ 1.077 barrier. Nycoprep™ 1.077, which is normally used for the purification of human peripheral blood mononuclear cells, is still available commercially (see Table 1)

3. Nycodenz® density gradient fractionation

There are such a variety of published pre-gradient operations, density gradient conditions and gradient separation characteristics that selection of one methodology would not be useful. Instead some of the centrifugation protocols and their fractionation characteristics are summarized in Table 1.

Table 1 Some examples of the use of Nycodenz® gradients for the purification of different forms of parasite

Parasite ¹	Separation of (temperature):	Nycodenz®	RCF/time	Ref
<i>P. falcip.</i>	Gametocytes (37°C) or ookinetes (25°C)	12%	1500g/15min	1
	Macrogametes/zygotes @ 6%/11% interface (23°C)	6%,11%,16%	16000g/10min	3
	Macrogametes/zygotes @ 6%/11% interface; non-activated gametocytes @ 11%/16% interface; pellet – asexual parasites and uninfected erythrocytes (4°C)	6%,11%,16%	7000g/30min ²	4
	Female gametes/zygotes from cultured mature gametocytes	6%,11%,16%	7000g/30min ³	5
	Non-activated gametocytes/macrogametes	6%,11%,16%	7000g/30min ³	6
	Gametes @ 6%/11%; gametocytes (stages II-IV) @ 11%/16%	6%,11%,16%	7000g/30min ³	7
	Infected erythrocytes (synchronized by sorbitol)	6%,11%,16%	16000g/10min ⁴	17
	Schizont infected erythrocytes (20°C)	16% ⁵	400g/20 min	22
	Extracellular gametes	6%,11%,16%	16000g/10min ⁴	18
	Gametes and zygotes	6%,11%,16%	7000g/30min ³	19
	Gametocytes (24°C)	14.1% ⁶	1900g/30 min	25-27
<i>P. berghei</i>	Removal of lysed erythrocytes/blood cells/debris (20°C)	17%	3000g/30min	9
	Ookinete purification (20°C)	17%	3000g/30min	10
	Ookinete purification (20°)	17%	3000g/30min	11
	Ookinetes from uninfected erythrocytes (20°C)	17%	1600g/30min	12
	Mosquito mid-gut parasites (4°C)	19%	not stated	13
	Gametocytes/uninfected/ring-infected erythrocytes (37°C) ⁷	13.25%	200g/25 min	14
	Gametocytes	13.25%	200g/25 min	16
	Ookinetes/depletion of erythrocytes	12%	3000g/30min	20
	Gametocyte-infected erythrocytes	15%	500g/25min	24
<i>P. reichen.</i>	Mature parasites	16%	400g/20 min	15
<i>P. yoelii</i>	Schizonts (20°C)	16.5%	300g/25 min	21
	Schizonts and trophozoites	14,16.5,19.3%	not given	28,29
	Schizonts and trophozoites	15.2%,22%	not given	30
	Parasitized erythrocytes	14%	400g/20 min	23

¹ *falcip.* = *falciparum*, *reichen.* = *reichenowi*

² Slow acceleration program used

³ Details of centrifugation not given in paper (ref 4 conditions are implied)

⁴ Details of centrifugation not given in paper (ref 3 conditions are implied)

⁵ 2.5 vol. of sample over 1 vol. of density barrier

⁶ Nycoprep 1.077™ is used as the source of this density barrier

⁷ Temperature chosen to prevent activation of gametocytes

4. Use of OptiPrep™

It is very likely that iodixanol can be substituted for Nycodenz® in these applications. Certainly the availability of iodixanol as a 60% (w/v) solution (OptiPrep™) makes gradient solution preparation much easier than is the case with Nycodenz®. Iodixanol and Nycodenz® solutions of the same % (w/v) concentration have almost identical densities, but solutions of Nycodenz® are hyperosmotic above 1.15 g/ml, in contrast to those of iodixanol which can be made isoosmotic at all densities. Whether the osmolality of Nycodenz® solutions plays an important role in achieving the separations described in this Application Sheet is not known. Comparisons can only be made empirically. For the preparation of iodixanol gradient solutions see [Application Sheet C01](#).

Janse et al [31] described detailed protocols for these organisms. The standard stock solution of Nycodenz® was a 1.15 g/ml solution containing 5 mM Tris-HCl, 0.3 mM Ca/Na₂ EDTA and 3 mM KCl, which was diluted 1:1 with PBS. 30 ml of schizont-containing blood was underlaid with 10 ml of this medium and centrifuged at 450 g for 20 min to band the schizonts at the interface. The authors also indicated that Nycodenz® was considerable less toxic to schizonts than Percoll®. A later paper [32] also reported that Nycoprep™ 1.077A (no longer commercially available) or OptiPrep™ might be used for the isolation of mature schizonts from cultures. The method for the preparation of this solution is

described in **OptiPrep™ Application Sheet C43**. Note however that these two solutions were primarily designed for rodent blood mononuclear cell isolation and are slightly hypoosmotic. The solutions described in Table 1 of **Application Sheet C01** are isoosmotic.

More recently *Plasmodium falciparum* [33,34] and *Plasmodium berghei* have been purified in iodixanol gradients [33,35]. Cha et al [35] described the use of a two-layer gradient of 15.4 and 10.2% iodixanol centrifuged at 16,500 g for 10 min. The organisms banded at the top of the lower density layer.

5. References

1. Carter, E.H., Suhrbier, A., Beckers, P.J.A. and Sinden, R.E. (1987) *The in vitro cultivation of P. falciparum ookinetes, and their enrichment on Nycodenz gradients* Parasitology, **95**, 25-30
2. Dearsly, A.L., Nicholas, J. and Sinden, R.E. (1987) *Sexual development in Plasmodium berghei: the use of mitomycin C to separate infective gametocytes in vivo and ookinetes in vitro* Int. J. Parasitol., **17**, 1307-1312
3. Quakyi, I.A., Carter, R., Rener, J., Kumar, N., Good, M.F. and Miller, L.H. (1987) *The 230 kDa gamete surface protein of Plasmodium falciparum is also a target for transmission-blocking antibodies* J. Immunol., **139**, 4213-4217
4. Vermeulen, A.N., Ponnudurai, T., Beckers, P.J.A., Verhave, J-P., Smits, M.A. and Meuwissen, J.H.E.T. (1985) *Sequential expression of antigens on sexual stages of Plasmodium falciparum accessible to transmission-blocking antibodies in the mosquito* J. Exp. Med., **162**, 1460-1476
5. Contreras, C.E., Ploton, I.N., Siliciano, R.F., Karp, C.L., Viscidi, R. and Kumar, N. (1998) *Mapping of specific and promiscuous HLA-DR-restricted T-cell epitopes on the Plasmodium falciparum 27-kilodalton sexual stage-specific antigen* Infect. Immun., **66**, 3579-3590
6. Dechering, K.J., Thompson, J., Dodemont, H.J., Eling, W. and Konings, R.N.H. (1997) *Developmentally regulated expression of pfs 16, a marker for sexual differentiation of the human malaria parasite Plasmodium falciparum* Mol. Biochem. Parasitol., **89**, 235-244
7. Healer, J., Graszynski, A. and Riey, E. (1999) *Phagocytosis does not play a major role in naturally acquired transmission-blocking immunity to Plasmodium falciparum malaria* Infect. Immun., **67**, 2334-2339
8. Mons, B., Croon, J.J.A.B., van der Star, W. and van der Kaay, H.J. (1988) *Erythrocytic shizogony and invasion of Plasmodium vivax in vitro* Int. J. Parasitol., **18**, 307-311
9. Al-Layan, E.M., Williams, G.T. and Hurd, H. (2002) *Apoptosis in the malaria protozoan, Plasmodium berghei: a possible mechanism for limiting intensity of infection in the mosquito* Int. J. Parasitol., **32**, 1133-1143
10. Arrighi, R.B.G. and Hurd, H. (2002) *The role of Plasmodium berghei ookinete proteins in binding to basal lamina components and transformation into oocysts* Int. J. Parasitol., **32**, 91-98
11. Blanco, A.R.A., Paez, A., Gerold, P., Dearsly, A.L., Margos, G., Schwarz, R.T., Barker, G., Rodriguez, M.C. and Sinden, R.E. (1999) *The biosynthesis and post-translational modification of Pbs21 an ookinete-surface protein of Plasmodium berghei* Mol. Biochem. Parasitol., **98**, 163-173
12. Carter, V., Cable, H.C., Underhill, B.A., Williams, J. and Hurd, H. (2003) *Isolation of Plasmodium berghei ookinetes in culture using Nycodenz density gradient columns and magnetic isolation* Malaria J., **2**:35
13. Dessens, J.T., Beetsma, A.L., Dimopoulos, G., Wengelnik, K., Crisanti, A., Kafatos, F.C. and Sinden, R.E. (1999) *CTRP is essential for mosquito infection by malaria ookinetes* EMBO J., **18**, 6221-6227
14. Janse, C.J. and Waters, A.P. (1995) *Plasmodium berghei: the application of cultivation and purification techniques to molecular studies of malaria parasites* Parasitol. Today, **11**, 138-143
15. Kocken, C.H.M., van der Wel, A., Dubbeld, M.A., Narum, D.L., van de Rijke, F.M., van Gemert, G-J., van der Linde, X., Bannister, L.H., Janse, C., Waters, A.P., and Thomas, A.W. (1998) *Precise timing of expression of a Plasmodium falciparum-derived transgene in Plasmodium berghei is a critical determinant of subsequent subcellular localization* J. Biol. Chem., **273**, 15119-15124
16. Kocken, C.H.M., Narum, D.L., Massougbodji, A., Ayivi, B., Dubbeld, M.A., van der Wel, A., Conway, D.J., Sanni, A. and Thomas, A.W. (2000) *Molecular characterization of Plasmodium reichenowi apical membrane antigen-1 (AMA-1), comparison with P. falciparum AMA-1, and antibody-mediated inhibition of red cell invasion* Mol. Biochem. Parasitol., **109**, 147-156
17. Kongkasuriyachai, D., Fujioka H. and Kumar, N. (2004) *Functional analysis of Plasmodium falciparum parasitophorous vacuole membrane protein (pfs16) during gametocytogenesis and gametogenesis by targeted gene disruption* Mol. Biochem. Parasitol., **133**, 275-285
18. Kumar, N. (1997) *Protein phosphorylation during sexual differentiation in the malaria parasite Plasmodium falciparum* Mol. Biochem. Parasitol., **87**, 205-210
19. Lobo, C.A., Dhar, R. and Kumar, N. (1999) *Immunization of mice with DNA-based Pfs25 elicits potent malaria transmission-blocking antibodies* Infect. Immun., **67**, 1688-1693
20. Margos, G., Navarette, S., Butcher, G., Davies, A., Willers, C., Sinden, R.E. and Lachmann, P.J. (2001) *Interaction between host complement and mosquito-midgut-stage Plasmodium berghei* Infect. Immun., **69**, 5064-5071
21. Mota, M.M., Thathy, V., Nussenzweig, R.S. and Nussenzweig, V. (2001) *Gene targeting in the rodent malaria parasite Plasmodium yoelii* Mol. Biochem. Parasitol., **113**, 271-278
22. Narum, D.L. and Thomas, A.W. (1994) *Differential localization of full-length and processed forms of PF83/AMA-1 an apical membrane antigen of Plasmodium falciparum merozoites* Mol. Biochem. Parasitol., **67**, 59-68
23. Narum, D.L., Ogun, S.A., Thomas, A.W. and Holder, A.A. (2000) *Immunization with parasite-derived apical membrane antigen 1 or passive immunization with a specific monoclonal antibody protects BALB/c mice against lethal Plasmodium yoelii YM blood-stage infection* Infect. Immun., **68**, 2899-2906

24. Rodriguez, M.C., Margos, G., Compton, H., Ku, M., Lanz, H., Rodriguez, M.H. and Sinden, R.E. (2002) *Plasmodium berghei*: routine production of pure gametocytes, extracellular gametes, zygotes, and ookinetes *Exp. Parasitol.*, **101**, 73-76
25. Fivelman, Q.L., McRobert, L., Sharp, S., Taylor, C.J., Saeed, M., Swales, C.A., Sutherland, C.J. and Baker, D.A. (2007) *Improved synchronous production of Plasmodium falciparum gametocytes in vitro* *Mol. Biochem. Parasitol.*, **154**, 119-123
26. Leber, W., Skippen, A., Fivelman, Q., Bowyer, P.W., Cockroft, S. and Baker, D.A. (2009) *A unique phosphatidylinositol 4-phosphate 5-kinase is activated by ADP-ribosylation factor in Plasmodium falciparum* *Int. J. Parasitol.*, **39**, 645-653
27. Schnick, C., Polley, S.D., Fivelman, Q.L., Ranford-cartwright, L.C., Wilkinson, S.R., Branningham, J.A., Wilkinson, A.J. and Baker, D.A. (2009) *Structure and non-essential function of glycerol kinase in Plasmodium falciparum blood stages* *Mol. Microbiol.*, **71**, 533-545
28. Fonager, J., Cunningham, D., Jarra, W., Koernig, S., Henneman, A.A., Langhorn, J. and Preiser, P. (2007) *Transcription and alternative splicing in the yir multigene family of the malaria parasite Plasmodium y. yoelii: Identification of motifs suggesting epigenetic and post-transcriptional control of RNA expression* *Mol. Biochem. Parasitol.*, **156**, 1-11
29. Ramalingam, J.K., Hunke, C., Gao, X., Grüber, G. and Preiser, P.R. (2008) *ATP/ADP Binding to a Novel Nucleotide Binding Domain of the Reticulocyte-binding Protein Py235 of Plasmodium yoelii* *J. Biol. Chem.*, **283**, 36386-36396
30. Cunningham, D., Fonager, J., Jarra, W., Carret, C., Preiser, P. and Langhorne, J. (2009) *Rapid changes in transcription profiles of the Plasmodium yoelii yir multigene family in clonal populations: lack of epigenetic memory?* *PLoS One*, **4**, e4285 (2009)
31. Janse, C.J., Ramesar, J. and Waters, A.P. (2006) *High-efficiency transfection and drug selection of genetically transformed blood stages of the rodent malaria parasite Plasmodium berghei* *Nat. Protoc.*, **1**, 346-356
32. Wykes, M.N., Kay, J.G., Manderson, A., Liu, X.Q., Brown, D.L., Richard, D.J., Wipasa, J., Jiang, S.H., Jones, M.K., Janse, C.J., Waters, A.P., Pierce, S.K., Miller, L.H., Stow, J.L. and Good, M.F. (2011) *Rodent blood-stage Plasmodium survive in dendritic cells that infect naive mice* *Proc. Natl. Acad. Sci. USA*, **108**, 11205-11210
33. Ma, J., Trop, S., Baer, S., Rakhmanaliev, E., Arany, Z., Dumoulin, P., Zhang, H., Romano, J., Coppens, I., Levitsky, V. and Levitskaya, J. (2013) *Dynamics of the major histocompatibility complex class I processing and presentation pathway in the course of malaria parasite development in human hepatocytes: implications for vaccine development* *PLoS One*, **8**: e75321
34. Hain, A.U.P., Bartee, D., Sanders, N.G., Miller, A.S., Sullivan, D.J., Levitskaya, J., Freel Meyers, C. and Bosch, J. (2014) *Identification of an Atg8-Atg3 protein-protein interaction inhibitor from the Medicines for Malaria Venture Malaria Box active in blood and liver stage Plasmodium falciparum parasites* *J. Med. Chem.* 2014, **57**, 4521-4531
35. Cha, S-J., Park, K., Srinivasan, P., Schindler, C.W., van Rooijen, N., Stins, M. and Jacobs-Lorena, M. (2015) *CD68 acts as a major gateway for malaria sporozoite liver infection* *J. Exp. Med.*, **212**, 1391-1403