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, theses 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
P. falcip.	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
P. berghei	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
P. reichen.	Mature parasites	16%	400g/20 min	15
P. yoelii	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/Na_2 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 an iodixanol

solution of similar density and osmolality to Nycoprep™ 1.077A is described **in OptiPrep™ Application Sheet C43**. 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

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Application Sheet C41; 7th edition, February 2018

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