

# Nycodenz® Application Sheets

## C41 Purification of malarial parasites (*Plasmodium falciparum*, *Plasmodium berghei*, *Plasmodium vivax*, and *Plasmodium yoelii*)

### 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 Nycodenz® Application Sheet.

As far as we know, iodixanol has not been used for fractionation of any form of *Plasmodium*, but that is not to say that it would not be as effective as Nycodenz®.

### 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.

OptiPrep™, a sterile 60% (w/v) of iodixanol can be diluted directly with a balanced salt solution, buffered saline solution or culture medium to produce solutions of lower density; these solutions will also be approx. isoosmotic with mammalian plasma. Solutions of Nycodenz® and iodixanol of the same % (w/v) concentration have a very similar density.

### 3. 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 <sup>1</sup>	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 <sup>2</sup>	4
	Female gametes/zygotes from cultured mature gametocytes	6%,11%,16%	7000g/30min <sup>3</sup>	5
	Non-activated gametocytes/macrogametes	6%,11%,16%	7000g/30min <sup>3</sup>	6
	Gametes @ 6%/11%; gametocytes (stages II-IV) @ 11%/16%	6%,11%,16%	7000g/30min <sup>3</sup>	7
	Infected erythrocytes (synchronized by sorbitol)	6%,11%,16%	16000g/10min <sup>4</sup>	17
	Schizont infected erythrocytes (20°C)	16% <sup>5</sup>	400g/20 min	22
	Extracellular gametes	6%,11%,16%	16000g/10min <sup>4</sup>	18
<i>P. berghei</i>	Gametes and zygotes	6%,11%,16%	7000g/30min <sup>3</sup>	19
	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 and ring-infected erythrocytes (37°C) <sup>6</sup>	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
	Parasitized erythrocytes	14%	400g/20 min	23

<sup>1.</sup> *falcip.* = *falciparum*, *reichen.* = *reichenowi*

<sup>2.</sup> Slow acceleration program used

<sup>3.</sup> Details of centrifugation not given in paper (ref 4 conditions are implied)

<sup>4.</sup> Details of centrifugation not given in paper (ref 3 conditions are implied)

<sup>5.</sup> 2.5 vol. of sample over 1 vol. of density barrier

<sup>6.</sup> Temperature chosen to prevent activation of gametocytes

#### 4. Literature summary

In addition to the references cited in Table 1, there are many other papers that report the use of Nycodenz® in a variety of studies on *Plasmodium berghei* (25-40), *Plasmodium vivax* (25,41,42) *Plasmodium yoelii* (43,44) and *Plasmodium chabaudi* (45-48). The majority of studies are however on *Plasmodium falciparum* and these studies include those on surface antigens (49-56), modulation of infectivity and transmission (57-60), gene regulation and expression (61-64), interactions with and properties of erythrocytes and reticulocytes (65-67), the actin gene (68-70), DNA synthesis (25),  $\alpha$ -tubulin (71,72), growth cycle (73,74), CO<sub>2</sub> fixation (75), culture (76) and disease diagnosis (77).

#### 5. References

To access abstracts of refs (file CA41) click on the double blue arrow →→

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