

Technical Note

TN2005-03

Technique: Density gradient isolation of cynomolgus (*M. fascicularis*) PBMC

Description: Cynomolgus monkey PBMCs require conditions for isolation that differ from human or rhesus macaque. Using standard ficoll-hypaque density gradients, the mononuclear cell 'buffy coat' from cynos will typically contain unacceptable contamination by RBCs and granulocytes. We found that purity of the mononuclear cell layer could be improved if the density of standard ficoll-hypaque was reduced by dilution with PBS. Using 90-95% ficoll hypaque, we could get 84-91% mononuclear cells while maintaining cell yield (Table 1). At these concentrations, the RBC contamination was acceptable.

Our standard protocol for isolating PMBC from cynomolgus monkeys now uses 90% ficoll-hypaque with centrifugation at 2,000G for 30 minutes.

Table 1. Isolation of cynomolgus PBMC from whole blood

Percent Ficoll	Yield^a Mean (range)	Purity (% MNCs)^b Mean (range)	RBC contamination
100	14.1 (10.7-17.1)	79 (70-87)	Severe
95	15.9 (11.0-22.6)	87 (84-91)	Moderate
➔ 90	12.6 (9.2-18.1)	90 (87-91)	Slight
85	6.6 (3.8-7.4)	87 (83-93)	Negligible

^a Nucleated cells (millions) harvested from 2.5 ml blood

^b Mononuclear cells as determined by ADVIA120 hematology analyzer

However, after using this method in a large number of animals, we find that some individual macaques differ in the optimal concentration needed to for pure PBMC isolation. While a 90% ficoll-hypaque is acceptable for most animals, some will isolate better at 85% or 95%. In some cases, it might be worthwhile to determine the optimal density for each individual animal.

Keith Reimann
20 February 2003
05 August 2006 revised



NIH NONHUMAN PRIMATE REAGENT RESOURCE

<http://nhpreagents.bidmc.harvard.edu>
Beth Israel Deaconess Medical Center
Center for Life Science 1038
330 Brookline Avenue
Boston, MA 02215
617-735-4459