

FICOLL ISOPAQUE TECHNIQUE

Ficoll-Isopaque Reagent

1. 6.48gm Ficoll + 10.17 gm Isopaque dissolved in 90 ml H₂O, then q.s. to 102 ml
Lasts at least one month. d = 1.077gm/cc
2. 33.9% Isopaque (density = 1.2000) (determine by weighing 100 ml or 50 ml)
9.0% Ficoll (density = 1.025)

Add 10 parts Isopaque to 24 parts Ficoll & check density = 1.077 gm/ml

Technique

1. Warm Ficoll-Isopaque to room temp.
2. Dilute cell sample to 1/2 cell density of blood*
*blood should be defibrinated with stirring and glass beads for 7 minutes before diluting
3. Layer sample < 20 ml carefully over 10 cc Ficoll-Iso at room temp
< 10 ml " " 5 cc Ficoll-Iso at room temp
4. Centrifuge at room temp for 35 min at 400g (Clinical IEC use setting #6)
5. Withdraw lymphocyte layer with Pasteur pipet
6. Dilute sample 1:4 in DsPBS and mix well.
7. Centrifuge in cold at 290g.

15ml blood yields $\sim 2.5 \times 10^7$ lymphs

Boyum 1967 Scand Journal of Clinical Lab Invest 21:Suppl 97:77