

**Procedure Title: Preparation of Goat Anti-Mouse Antibody for Use in Coating Magnetic Beads**

**Procedure #: HSC.B203.01**

1. Reagents, Supplies, Equipment
  - A. Goat affinity purified antibody (GAM) IgG
  - B. Goat affinity purified antibody (GAM) IgM
  - C. PBS-1X sterile and pyrogen free
  - D. 2 ml sterile cryovials
  - E. Nalgene filter units (150ml)
  - F. 25 ml pipets
  - G. Sterile pipets
  - H. Graduated cylinder
  
2. Procedure
  - A. Add 2.0 ml of 1X PBS/vial of affinity purified goat anti mouse antibody. Use equal (mg) amounts of IgG and IgM.
  - B. Warm the vials in 37<sup>0</sup>C water bath for 45 minutes.
  - C. Remove vials from bath and cool slowly to room temperature. Combine the contents of all vials and rinse vials once with sterile 1x PBS.
  - D. Take IgG IgM antibody solution to a volume which will give a concentration of 2.5 mg/ml (1.25 mg/ml IgG and 1.25 mg/ml IgM).
  - E. If the solution is cloudy use a pre-filter before sterile filtration.
  - F. Using good sterile technique in a laminar flow hood, filter antibody solution

through a 0.2 $\mu$  filter.

- G. Pipet 1.0 ml aliquots into labelled 2 ml cryovials, and store vials in liquid nitrogen vapor until needed. The box must be labelled "NOT TESTED" or stored in a non BMT laboratory until the batch has passed sterility and pyrogen testing.

Label information:

Date

Sterile GAM# (# = Batch number )

- H. Send the requisite number of vials (determined by USP guidelines) along with the necessary forms to Currie labs for sterility and pyrogen testing.

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- I. Send a vial to CHLA Microbiology Dept. for routine sterility testing.