

**Procedure Title: Preparation of Monoclonal Antibody Coated Beads**

**Procedure #: HSC.B202.01**

1. Principle

Magnetic beads used in Neuroblastoma tumor purging must be washed, coated with goat anti- mouse IgG and IgM antibodies which ( adsorb to the plastic surface of the beads ), and finally incubated with Neuroblastoma specific monoclonal antibodies which bind to the GAM.

2. Reagents, Supplies, Equipment

- A. Dynal M450 uncoated magnetic beads
- B. PBS-1X
- C. GAM - Goat anti mouse IgG and IgM (see the GAM prep protocol); sterile in PBS at 2.5 mg/ml.
- D. Magnetic separator device
- E. Rotator
- F. Sonicator
- G. 225 ml sterile conical polypropylene centrifuge tubes
- H. Monoclonal antibodies
- I. Leibovitz media (L-15)- Irvine Scientific #9083
- J. Gentamicin
- K. Fetal calf serum ( heat Inactivated at 60 °C for one hour- lot tested and certified sterile )

3. Procedure

All the steps below except sonication, centrifugation, and cold room rotation are to be done in a laminar flow hood. Beads must be prepared at least fourteen days before the harvest so that they can be tested for sterility and tumor removal before being used.

- A. Dispense 90 ml of (ethanol washed) M-450 Dynal beads (in water at  $\sim 4 \times 10^8$  beads/ml into a sterile 225 ml conical tube.
- B. Wash the beads three times by suspending the beads in PBS-1X, centrifuging at 490xg for 10 min., and removing the liquid by aspiration.
- C. Add 12.5 mg GAM to 90 ml of beads (2.5 mg for 18 ml of beads) and take to  $\sim 250$  ml final volume in PBS-1X for ( $\sim 50$ ug/ml) of GAM.

- D. Cap the tube, tighten securely, wrap cap with parafilm, and rotate overnight or a minimum of four hours at 4<sup>0</sup>C. If these beads are to be used for indirect purging they should be kept in coating buffer until they have to be tested and used.
- E. Wash the beads 3 times as in step B, resuspend to ~250ml and wrap the cap with parafilm.
- F. Rotate the beads for 1 hour at 4<sup>0</sup>C in PBS-1X.
- G. Wash the beads 2X with PBS-1X.
- H. Add monoclonal antibodies: 12.5 mg of IgG and 25.0 mg of IgM to the beads and take to ~250 ml in PBS-1X for a final concentration of 50 ug/ml of IgG and 100 ug/ml of IgM.
- I. Rotate the beads for a minimum of 3 hours or overnight at 4<sup>0</sup>C.
- J. Wash the antibody coated beads two times with L15 and FCS to 10% v/v as in step B. Rotate for one hour at 4<sup>0</sup> C and wash twice again in L15 and FCS to 10% v/v .
- K. After the second wash resuspend the beads in L15/FCS to 180 ml. Remove aliquots for sterility and tumor removal testing. Aliquot 20 ml ( 4 x10<sup>9</sup> beads ) into sterile 50 ml tubes. Wrap the caps with parafilm and label the tubes with the following information:
  - a. Date coated with GAM
  - b. Dated coated with monoclonal antibodies
  - c. Monoclonal antibody cocktail
  - d. Date of bead test
  - e. Number the tubes 1-1 to 1-9 and 2-1 to 2-9
- L. Record all pertinent information on the Immunobead Worksheet.