

BioCapture LLC™
Goat Anti-Mouse (GAM) IgG
Coupled Particles (CAP)

GAM-CAP Package Insert

| Product Number: C00001 (1 mL); C00002 (2 mL)
Shelf Life: One Year

NOTE: For research use only. Not for use in human diagnostic or therapeutic procedures.

IMPORTANT: DO NOT ALLOW PARTICLES TO COME IN CONTACT WITH A MAGNETIC FIELD UNTIL DIRECTED TO DO SO IN THE PROTOCOL.

This product is to be used to purge unwanted cells from a cell suspension. The desired cells recovered after purging will be close to 100% yield. See our website: www.biocapturellc.com for more details.

Cell Purging Protocols

A. Preincubation of mouse IgG monoclonal antibodies with GAM-CAPs

1. Rinse cells (up to 5×10^6 total cells/ml) using procedures standard in your laboratory. Resuspend in cell buffer or PBS/0.1 % BSA/pH 7.2.
2. Vortex GAM-CAPs bottle well before removing particles. Rinse as instructed in “CAPs Rinsing Procedures” below. Resuspend GAM-CAPs to the original volume.
3. Add sufficient monoclonal antibody to the GAM-CAPs* for depletion of the target cell population (note: The quantity of antibody required will depend upon the number of cells, the antigen density, the monoclonal antibody affinity, and should be determined by the researcher). Incubate antibody with GAM-CAPs by vortexing for 1-2 minutes (sample volume ≤ 2 ml) or end-over-end mixing for 4 minutes (sample volume > 2 mL).
4. Following incubation, wash particles three times with Cell Buffer or PBS/0.1% BSA/pH 7.2 by vortexing or end-over-end mixing for 1 minute each wash.
5. Vortex the GAM-CAPs/monoclonal antibody particles well and add (immediately to cells (50 uL particles/ml cell suspension)).
6. Immediately vortex (sample volume ≤ 2 ml) for 1-2 minutes or end-over-end mix (sample volume > 2 mL) for 4 minutes (Equipment 1 below).

7. Immediately place in the appropriate magnetic separator (Equipment 2 below) for 30 seconds.
8. Carefully remove the sample while still in the magnetic field to obtain the desired depleted sample.

*This amount of GAM-CAPs/ml is recommended as a starting point. The actual amount of particles/ml, mixing time and/or magnetic separation time should be varied to determine the best parameters for the cell type/mab(s) used.

B. Preincubation of mouse IgG mouse monoclonal antibodies with cells

1. Label cells with desired mouse IgG monoclonal antibodies, rinse and resuspend cells (up to 5×10^6 total cells/ml) using procedures that are standard in your laboratory. Resuspend cells in Cell Buffer or PBS/0.1 %BSA/pH 7.2.
2. Vortex bottle containing GAM-CAPs well before removing particles. Rinse as instructed in "CAPs Rinsing Procedures" below. Resuspend GAM-CAPs to the original volume.
3. Vortex GAM-CAPs particles well and add 50 ul/ml cell suspension*.
4. Immediately mix either by vortexing (sample volume ≤ 2 ml) for 1-2 minutes or end-over-end mixing (sample volume > 2 mL) for 4 minutes (Equipment 1 below).
5. Immediately place in the appropriate magnetic separator (Equipment 2 below) for 30 seconds.
6. Carefully remove the sample while still in the magnetic field to obtain the desired depleted sample.

*This amount of GAM-CAPs/ml is recommended as a starting point. The actual amount of particles/ml, mixing time and/or magnetic separation time should be varied to determine the best parameters for the cell type/ mab(s) used.

CAPs Rinsing Procedures

GAM-CAPs can be rinsed in three different ways: 1. centrifugation, 2. gravity settling or 3. magnetic field exposure. Procedure 1 or 2 is recommended. If rinsing using magnetic separation, particles must be demagnetized prior to use.

Rinsing Buffer PBS/0.1%BSA/pH 7.2.

Note: Particles should not be stored in rinsing buffer.

Final resuspension buffer can be Rinse Buffer or Cell Buffer.

1. Centrifugation: Vortex bottle well before removing desired quantity of GAM-CAPs. Centrifuge at 500 rpm for 30 seconds, remove buffer; add fresh buffer; resuspend particles by vortexing or pipette up and down. Repeat two times. Resuspend to original volume of Rinse Buffer or Cell Buffer. .

2. Gravity Settling: Vortex bottle well before removing desired quantity of GAM-CAPs. Allow particles to settle by gravity for 2-3 minutes; remove buffer; add fresh buffer; resuspend particles by vortexing or pipette up and down. Repeat two times. Resuspend to original volume of Rinse Buffer or Cell Buffer.
3. Magnetic Separation: Vortex bottle well before removing desired quantity of GAM-CAPs. Place particles in magnetic separator for 4-5 seconds; remove buffer; remove test tube from magnetic separator; add buffer; resuspend by vortexing or pipette up and down. Repeat two times. Resuspend to original volume of Rinse Buffer or Cell Buffer. Demagnetize the final particle suspension.(see demagnetizer under Equipment)

Equipment required for optimal performance of GAM-CAPs:

1. Mixer: Due to the ~5 fold difference in density between GAM-CAPs and cells proper mixing is essential to ensure contact between the particles and the targeted cells. The bead cell mixture cannot be vortexed briefly and allowed to stand without mixing.
 - a. For reaction volumes < to 2.0 mL mixing can be accomplished by vortexing of a low speed using a Vortex Genie 2 with turbomixer attachment that has variable speed control. A Vortex Genie 2 with a timer is desirable but a timer is not essential.
 - b. For volumes > 2 mL up to 50ml mixing can be accomplished by end-over-end mixing using an ATR Rotomix mixer (www.atrbiotech.com/benchttop/rotomix.htm) with variable speed. Recommended mixing speed is 15-30 rpm.
2. Magnetic Separation:
 - a. Ideal magnets for use with GAM-CAPs can be obtained from Dexter Magnetic Technologies (www.lifesept.com under products). Different magnets are available for sample volumes from \leq 0.5mL to 50mL
 - b. Magnets from suppliers of superparamagnetic particles will also work with GAM-CAPs.
3. Demagnetizer: To ensure that particles are well dispersed, it is recommended, but not required unless particles are exposed to a magnetic field prior to use, that the particles be demagnetized immediately prior to the addition to the sample, using a demagnetizer/degausser from Data-Link Associates (www.datalinksales.com); product number PF-211.

Hold the demagnetizer at the bottom of the test tube containing the particles; (hold test tube in one hand; demagnetizer in the other hand; demagnetizer can touch test tube); turn on by holding the "on button" in the on position; rotate demagnetizer in a clockwise or counterclockwise motion for 10-12 seconds; WHILE UNIT IS STILL ON (if unit is turned off

before this step particles will be magnetized slowly move the demagnetizer away from the test tube (about 3 feet; arms length); turn off demagnetizer.

For technical information/Questions contact BioCapture LLC:

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