

Bio-Adembeads Antibodies Goat anti-Rat IgM M0513

For research use only

INTRODUCTION

Bio-Adembeads Antibodies Goat anti-Rat IgM were developed for the positive selection or depletion of cells labelled with primary Rat IgM antibodies. They can also be utilized for the positive selection or depletion of subcellular material, bacteria or other microorganisms labelled with primary Rat IgM antibodies.

PRODUCT DESCRIPTION

Bio-Adembeads Antibodies Goat anti-Rat IgM are monodispersed superparamagnetic particles coated with affinity purified Goat anti-Rat IgM covalently bound to the surface. These secondary antibodies are μ Chain Specific. Cross reactivity to Rat, bovine and Horse serum proteins is minimal. They are produced under aseptic conditions and are supplied in an aqueous suspension containing 0.05% Proclin 300.

PHYSICAL CHARACTERISTICS

Diameter: 300 nm (CV max 20%)

Magnetic susceptibility: approx. 40 emu/g

Specific surface area: 10 m²/g

Iron oxide content: approx. 70%

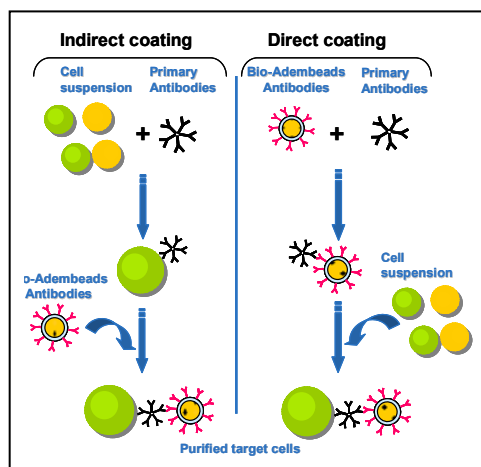
PRINCIPLE OF BIO ADEMBEADS ANTIBODIES SEPARATION

Indirect Coating

Cells are labeled with primary Rat IgM antibody. Subsequently, cells are rosetted with Bio-Adembeads Antibodies Goat anti-Rat IgM for a magnetically labeling.

Direct Coating

In this case the primary Rat antibody of the IgM-class is bound firstly to the Bio-Adembeads Antibodies Goat anti-Rat IgM before incubation with the cell suspension.



Note:

We recommended that you use indirect technique for cell separation and other formats of purification.

The type and amount of antibody required for optimal coating and performance of the beads will vary with the mAb-affinity and the antigen density on the surface. The optimal coating of beads to achieve an efficient binding of cells should be determined in each individual case by titration.

INSTRUCTIONS FOR USE

A) Indirect coating for cell separation

Before cells can be isolated with Bio-Adembeads Antibodies Goat anti-Rat IgM in the indirect technique, the cells must be incubated with specific Rat primary antibodies against surface markers on the target cells.

1. Incubate the heterogeneous cell suspension with the primary Rat IgM antibodies for 30 minutes at 2-8°C providing gentle mixing. Each primary antibody should be added in excess to occupy all antigen binding sites on the cell surface.

2. Collect the incubated cells by centrifugation at 800g for 10 minutes. Discard supernatant.

3. Resuspend and wash the incubated cells 2 times with Hanks Balanced Salt Solution, pH 7.4 to remove all unbound antibody. The cells are now covered with specific primary Rat antibodies. A cell concentration of 10-50 mill/ml sample is recommended.

4. Add minimum 30 μ l of Bio-Adembeads Antibodies Goat anti-Rat IgM per ml of primary antibody covered cells. (Titration ensures an optimal use of the Bio-Adembeads products)

5. Incubate for approx 15-30 minutes with titling and rotation mixer at 2-8°C.

6. Place the tube in a Adem-Mag SV or MV for 2-5 minutes and pipette off the supernatant. Alternatively, incubation cells medium can be added after the incubation to the height of the magnet to avoid trapping of non-bound cells.

7. Positively selected cells attached to the Bio-Adembeads should be washed four to five times with cells culture medium.

B) Direct coating for cell separation

1. Resuspend the Bio-Adembeads Antibodies Goat anti-Rat IgM by pipetting and vortexing. Avoid foaming.

2. Pipette the volume to be used into the desired test tube.

3. Place the tube in a magnet (see Related Product) for 1min.

4. Pipette off the supernatant carefully, leaving beads undisturbed.

5. Remove the test tube from the magnet (see Related Product) and resuspend the beads

carefully in the original sample volume with adequate buffer (Immobilisation Buffer recommended, see Related Product).

6. Resuspend thoroughly by vortexing and/or shaking.

7. Add maximum 1-5 μ g Rat IgM to 100 μ l of washed Bio-Adembeads Antibodies Goat anti-Rat IgM.

8. Incubate the mixture from 5 to 30 minutes at 20°C (incubation temperature depends on antibody stability) with titling and rotation for even mixing.

9. Place the tube in the magnet (Adem-Mag SV or MV, see related product) and leave to separate for 2 minutes.

10. Discard the supernatant while the tube is on the Adem-Mag magnet, taking care not to disturb the Adembeads. Remove the tube from the Adem-Mag magnet and resuspend the beads in the adequate buffer (Immobilisation Buffer). Mix gently for a few seconds, before applying the magnet again.

11. Repeat step 9-10 twice but the second time resuspend the beads in Hanks Balanced Salt Solution or incubation cells medium.

12. Incubate precoated Bio-Adembeads Antibodies Goat anti-Rat IgM and the heterogeneous cell suspension for approx. 15-30 minutes with titling and rotation at 2-8°C. Use a final Bio-Adembeads concentration suitable for your experiment.

13. Place the tube in a Adem-Mag SV or MV for 2-5 minutes and pipette off the supernatant. Alternatively, incubation cells medium can be added after the incubation to the height of the magnet to avoid trapping of non-bound cells.

14. Positively selected cells attached to the Bio-Adembeads should be washed four to five times with cells culture medium.