Datasheet for ABIN1305242

Anti-Human CD25 Magnetic Particles

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Overview

Quantity:	5 mL
Target:	CD25 (IL2RA)
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	Magnetic Particles
Application:	Separation (Sep)

Product Details

Brand:	BD IMag™

Target Details

Target:	CD25 (IL2RA)
Alternative Name:	CD25 (IL2RA Products)
Background:	BD IMag™ Anti-Human CD25 Magnetic Particles - DM are magnetic nanoparticles that have monoclonal antibody conjugated to their surfaces. These particles are optimized for the positive selection or depletion of CD25-bearing leukocytes using the BD IMagnet™. CD25 is the 55 kDa alpha chain of the IL-2 receptor that is expressed on activated B and T lymphocytes, which may also include regulatory T-cell (Treg cells). For enrichment of Treg cells, depletion of erythrocytes, platelets, monocytes, granulocytes and non-CD4 T lymphocytes is first recommended using the BD IMag™ Human CD4 T Lymphocyte Enrichment Set - DM, followed by the positive selection of the CD25+ population. Peripheral Blood Mononuclear Cells (PBMC)

Target Details

are labeled with BD IMag™ Anti-Human CD25 Magnetic Particles - DM according to the Magnetic Labeling and Separation Protocol. This labeled cell suspension is then placed within the magnetic field of the BD IMagnet™. Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off (negative fraction). The tube is then removed from the magnetic field for the resuspension of the positive fraction. The separation is repeated twice to increase the purity of the positive fraction. The magnetic separation steps are diagrammed in the Separation Flow Chart. After the positive fraction is washed, it can be further evaluated in downstream applications. The small size of the BD IMag™ particles allows the positive fraction to be further evaluated in downstream applications, such as by flow cytometry.

Application Details

Protocol:

1. Dilute BD IMag™ Buffer (10X) (Cat. No. 552362) 1:10 with sterile distilled water or prepare 1X BD IMag™ buffer by supplementing Phosphate Buffered Saline with 0.5% BSA, 2 mM EDTA, and 0.09% sodium azide. Store at 4°C.
2. Prepare PBMC from anti-coagulated human blood, preferably by density gradient centrifugation using Ficoll-Paque™. Optional: If Treg cells are desired, enrich the CD4 T lymphocytes by using the BD IMag™ Human CD4 T Lymphocyte Enrichment Set - DM (Cat. no. 557939).
3. Count the cells, wash them with an excess volume of 1X BD IMag™ buffer, and carefully aspirate all the supernatant.
4. Vortex the BD IMag™ Anti-Human CD25 Magnetic Particles - DM thoroughly, and add 50 µl of particles for every 10e7 total cells.
5. MIX THOROUGHLY. Incubate at room temperature for 30 minutes. Avoid nonspecific labeling by working quickly and adhering to recommended incubation times.
6. Bring the BD IMag™-particle labeling volume up to 1-8 x 10e7 cells/ml with 1X BD IMag™ buffer, and immediately place the tube on the BD IMagnet™. Incubate for 8 - 10 minutes.
7. With the tube on the BD IMagnet™, carefully aspirate off the supernatant. This supernatant contains the negative fraction.
8. Remove the tube from the BD IMagnet™, and add 1X BD IMag™ buffer to the same volume as in Step 6. Gently resuspend cells by pipetting up and down, and return the tube to the BD IMagnet™ for another 2 - 4 minutes.
9. With the tube on the BD IMagnet™, carefully aspirate off the supernatant and discard.
10. Repeat Steps 8 and 9.
11. After the final wash step, resuspend the positive fraction in an appropriate buffer or

Application Details

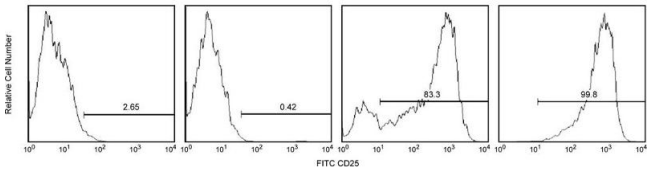
	medium, and proceed with desired downstream application(s). NOTES: Hints for successful cell preparation: Hints for successful cell preparation: a) Draw the blood into a tube containing EDTA (for example, BD Vacutainer EDTA tube, Cat. No. 366457 or 367661). b) Remove the platelet rich plasma by centrifuging once at 220-240 x g. c) Wash 2-3 times in PBS after the density gradient separation. d) Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	Aqueous buffered solution containing BSA and ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C
Storage Comment:	Store undiluted at 4°C.

Publications

Product cited in:	Curotto de Lafaille, Lafaille: "CD4(+) regulatory T cells in autoimmunity and allergy." in: **Current opinion in immunology**, Vol. 14, Issue 6, pp. 771-8, (2002) (PubMed).



Flow Cytometry

Image 1.