Technical Data Sheet

Human Regulatory T Lymphocyte Separation Set - DM

Product Information

Catalog Number: **558142**

Components: **Human Regulatory T Lymphocyte Separation Cocktail, 5.0 ml,** comprising of the following monoclonal antibodies:

- Biotin mouse anti-Human CD8, clone SK1
- Biotin mouse anti-Human CD11b/Mac-1 (CR3), clone ICRF44
- Biotin mouse anti-Human CD16, clone 3G8
- Biotin mouse anti-Human CD19, clone HIB19
- Biotin mouse anti-Human CD36, clone CB38 (NL07)
- Biotin mouse anti-Human CD41a, clone HIP8
- Biotin mouse anti-Human CD56, clone B159
- Biotin mouse anti-Human CD123 (IL-3 Receptor α chain), clone 9F5
- Biotin mouse anti-Human CD235a (Glycophorin A), clone GA-R2 (HIR2)
- Biotin mouse anti-Human γδ TCR, clone B1
- APC mouse anti-Human CD25, clone M-A251

Streptavidin Particles Plus – DM, 7.5 ml

Anti-APC Particles – DM, 3.0 ml

Storage Buffer: Aqueous buffered solution containing BSA* and 0.09% sodium azide.

Description

The BD IMag™ Human Regulatory T Lymphocyte Separation Set – DM is used for the isolation of $CD25^+$ $CD4^+$ regulatory T lymphocytes (Treg cells) from peripheral blood. The Human Regulatory T Lymphocyte Separation Cocktail contains biotinylated monoclonal antibodies (mAb) that recognize antigens expressed on erythrocytes, platelets, and peripheral leukocytes that are *not* CD4 T lymphocytes. It also contains anti–human CD25 mAb that is labeled with allophycocyanin (APC). After peripheral blood mononuclear cells (PMBC) are labeled with the cocktail, the $CD4^+$ $CD25^+$ Treg cells are isolated in two immunomagnetic separation steps. First, the Streptavidin Particles Plus – DM enrich the $CD4^+$ T lymphocytes by negative selection (depletion of the non-$CD4^+$ cells). In the second immunomagnetic separation step, the Anti-APC Particles – DM select the $CD25^+$ cells, from among the enriched $CD4^+$ cells, which already had been labeled with the APC anti-CD25 mAb. This Separation Set has been optimized for use with the BD IMagnet™, and it contains sufficient reagents to label 10^9 PBMC.

Storage

The monoclonal antibody cocktail should be protected from prolonged exposure to light and stored undiluted at 4°C. The streptavidin– and anti-APC–conjugated magnetic particles should be stored undiluted at 4°C. **Do not freeze.**

Usage

The detailed Magnetic Labeling and Separation Protocol follows. In summary, the Human Regulatory T Lymphocyte Separation Cocktail simultaneously labels erythrocytes, platelets, and most leukocytes except the CD4 T lymphocytes with biotin and $CD25^+$ cells with APC. After washing away excess antibody, the isolation of Treg cells is performed in two steps. First, Streptavidin Particles Plus – DM are added and bind to the cells bearing the biotinylated antibodies. The tube containing this labeled cell suspension is then placed within the magnetic field of the BD IMagnet™ (Cat. no. 552311). Negative selection is performed to enrich for the unlabeled CD4 T cells. Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off and retained (enriched fraction). The negative selection is repeated twice to increase the yield of the enriched fraction. To increase its purity, negative selection is then performed on the combined enriched fraction. Second, the $CD25^+$ Treg cells are isolated from the twice-enriched fraction by positive selection using the Anti-APC Particles – DM and the BD IMagnet™, which is repeated three times to increase the purity of the positive fraction. For clarification of the procedure, the magnetic separation steps are diagrammed in the Enrichment and Positive Selection Flow Charts. The twice-enriched fraction and the isolated Treg cells can be evaluated in downstream applications such as flow cytometry and tissue culture. The antibodies in the Human Regulatory T Lymphocyte Separation Cocktail have been optimized and pre-diluted to provide maximum efficiency for selection of Treg cells from PBMC.

BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.
**Source of all serum proteins is from USDA inspected abattoirs located in the United States.*

Hazardous Ingredient: Sodium Azide. *Avoid exposure to skin and eyes, ingestion, and contact with heat, acids, and metals. Wash exposed skin with soap and water. Flush eyes with water. Dilute with running water before discharge into plumbing.*

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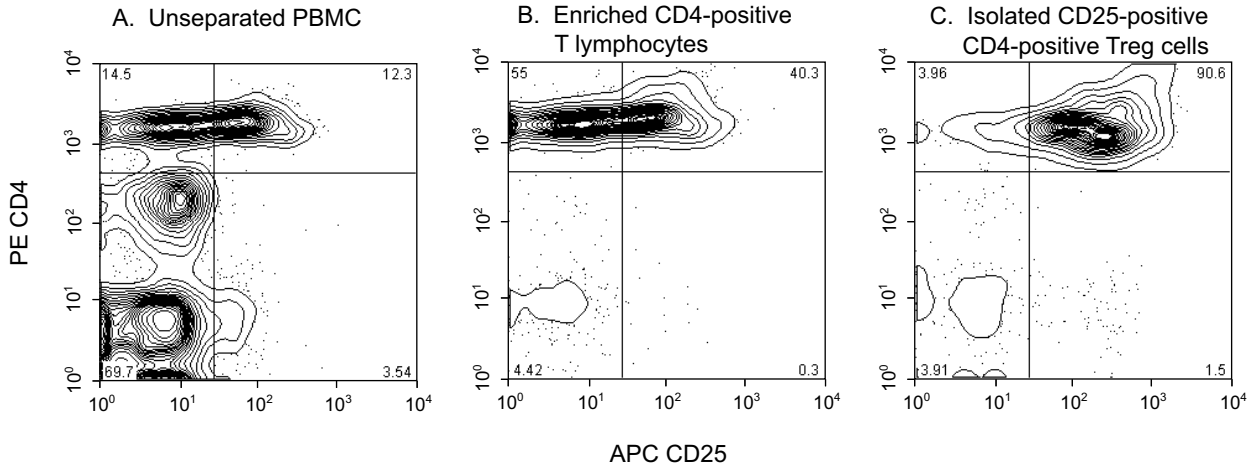

Isolation of Treg cells from human blood. *PBMC were labeled with the BD IMag™ Human Regulatory T Lymphocyte Separation Set - DM and separated on the BD IMagnet™ (Cat. no. 552311) according to the accompanying Protocol. To demonstrate the efficiency of the enrichment and selection steps, cells were stained with PE anti-human CD4 mAb RPA-T4 (Cat. no. 555347) and APC anti-human CD25 mAb M-A251 (Cat. no. 555432, Panel A only, because the cells in Panels B and C were already stained with the same reagent that is included in the Human Regulatory T Lymphocyte Separation Cocktail). Dead cells were excluded by staining with propidium iodide. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.*

Please refer to the Enrichment and Positive Selection Flow Charts on the next page to identify the cell populations represented in this figure. The percentage of $CD25^+$ $CD4^+$ T cells is indicated in the upper-right quadrant of each panel. Panel A shows unseparated PBMC, Panel B shows the twice-enriched CD4 T lymphocyte fraction, and Panel C shows the $CD4^+$ $CD25^+$ Treg cells after positive selection.

References

Shevach EM. Certified professionals: $CD4^+CD25^+$ suppressor T cells. *J. Exp. Med.* 2001;193:F41–F45.

Curotto de Lafaille MA, Lafaille JJ. $CD4^+$ regulatory T cells in autoimmunity and allergy. *Curr. Opinion in Immunol.* 2002;14:771–778.

Sakaguchi S. Naturally arising $CD4^+$ regulatory T cells for immunologic self-tolerance and negative control of immune responses. *Annu. Rev. Immunol.* 2004;22:531–562.

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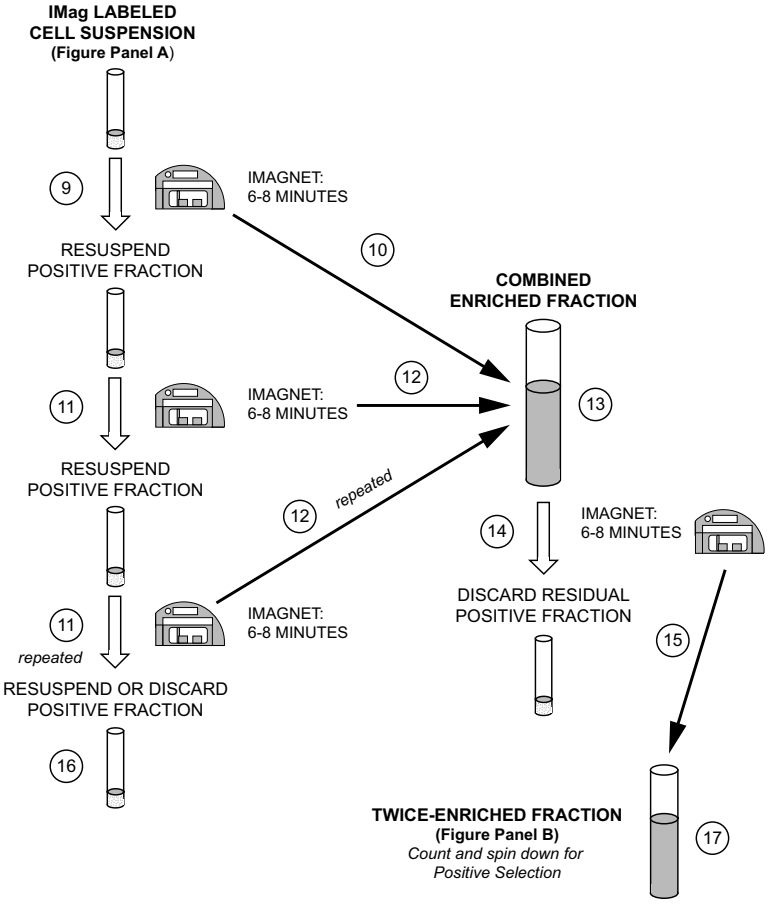
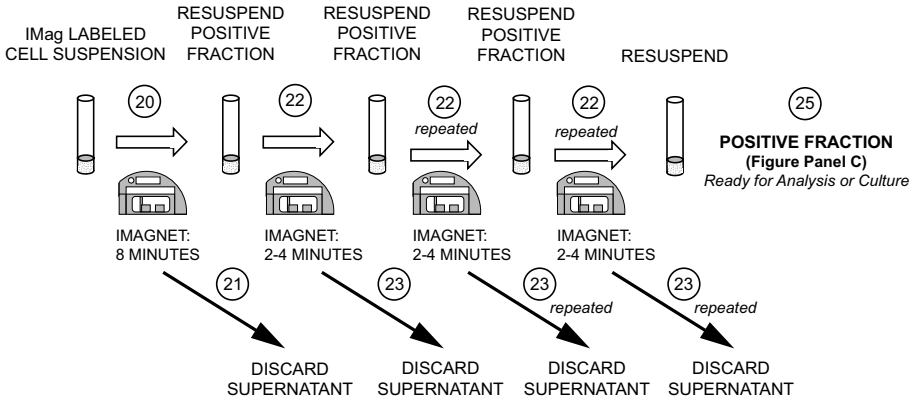
ENRICHMENT FLOW CHART

(The circled numbers correspond to the steps of the Protocol on the following page.)



POSITIVE SELECTION FLOW CHART

(The circled numbers correspond to the steps of the following Protocol.)



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MAGNETIC LABELING AND SEPARATION PROTOCOL

1. Prepare 1X BD IMag™ buffer: Dilute BD IMag™ Buffer (10X) (Cat. no. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline (PBS) supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.
2. Prepare PBMC from anticoagulated human blood, preferably by density gradient centrifugation using Ficoll-Paque™.*
3. Remove clumps of cells and/or debris by passing the suspension through a 70-μm nylon cell strainer. Count the cells, and centrifuge at 300 × *g* for 7 minutes (spin down).
4. Resuspend the cell pellet in the Human Regulatory T Lymphocyte Separation Cocktail at 5 μl per 1 × 10^6 cells, and incubate at room temperature for 15 minutes.†
5. Wash the labeled cells with a 10X excess volume of 1X BD IMag™ buffer, spin down, and carefully aspirate **ALL** the supernatant.

ENRICHMENT OF $CD4^+$ T LYMPHOCYTES

6. Vortex the BD IMag™ Streptavidin Particles Plus – DM thoroughly, and resuspend the cell pellet in 7.5 µl of the particles per 1 × 10^6 cells.
7. **MIX THOROUGHLY**. Incubate at room temperature for 30 minutes.†
8. Bring the labeling volume up to 20 to 80 x 10^6 cells/ml with 1X BD IMag™ buffer.
9. Transfer the labeled cells to a 12 × 75 mm round-bottom test tube (eg, BD Falcon™, Cat. no. 352058), maximum volume added not to exceed 1.0 ml. Place this positive-fraction tube on the BD IMagnet™ (horizontal position) for 6 to 8 minutes.
    - For greater volume, transfer the cells to a 17 × 100 mm round-bottom test tube (eg, BD Falcon™, Cat. no. 352057), maximum volume added not to exceed 3.0 ml. Place this positive-fraction tube on the BD IMagnet™ (vertical position) for 8 minutes.
10. With the tube on the BD IMagnet™ and using a sterile glass Pasteur pipette, carefully aspirate the supernatant (enriched fraction) and place in a new sterile tube.
11. Remove the positive-fraction tube from the BD IMagnet™, and add 1X BD IMag™ buffer to the same volume as in Step 8. Resuspend the positive fraction well by pipetting up and down 10 to 15 times, and place the tube back on the BD IMagnet™ for 6 to 8 minutes.
    - For 17 × 100 mm tube: Place on the BD IMagnet™ for 8 minutes.
12. Using a new sterile Pasteur pipette, carefully aspirate the supernatant and combine with the enriched fraction from Step 10 above.
13. Repeat steps 11 and 12. The combined enriched fraction contains CD4 T lymphocytes with no bound antibodies or magnetic particles.
14. Place the tube containing the combined enriched fraction on the BD IMagnet™ for another 6 to 8 minutes.
    - For 17 × 100 mm tube: Place on the BD IMagnet™ for 8 minutes.
15. Carefully aspirate the supernatant and place in a new sterile tube. This is the twice-enriched fraction containing CD4 T lymphocytes with no bound magnetic particles, that includes the $CD25^+$ $CD4^+$ cells already labeled with APC anti-CD25.
16. The positive-fraction cells remaining in the original tube can be resusupended in an appropriate buffer or culture medium for downstream applications, including flow cytometry, if desired.
17. Count the cells in the twice-enriched fraction, spin down, and carefully aspirate **ALL** the supernatant.

POSITIVE SELECTION OF $CD25^+$ $CD4^+$ CELLS

18. Vortex the BD IMag™ Anti-APC Particles – DM thoroughly, and resuspend the cell pellet in 5 μl of the particles per 1 × 10^6 cells.
19. **MIX THOROUGHLY.** Incubate at room temperature for 30 minutes.†
20. Bring the labeling volume up to 20 to 80 × 10^6 cells/ml with 1X BD IMag™ buffer, immediately place the tube onto the BD IMagnet™ and incubate for 8 minutes.
21. With the tube on the BD IMagnet™, carefully aspirate and discard the supernatant.
22. Remove the tube from the BD IMagnet™, and add 1X BD IMag™ buffer to the same volume as in Step 20. Gently resuspend the cells by pipetting up and down, and return the tube to the BD IMagnet™ for another 2 to 4 minutes.
23. With the tube on the BD IMagnet™, carefully aspirate and discard the supernatant.
24. Repeat Steps 22 and 23 twice.‡
25. After the final wash step, remove the tube from the BD IMagnet™. Resuspend the isolated Treg cells in an appropriate buffer or culture medium, and proceed with desired downstream application(s), including flow cytometry.
26. Samples of the total cell suspension, the twice-enriched fraction, and the isolated Treg cells should be analyzed by flow cytometry to evaluate the efficiency of the cell separation procedure.

* Hints for successful PBMC preparation:
- Draw the blood into a tube containing EDTA (for example, BD Vacutainer® EDTA tube, Cat. no. 366457 or 367661).
- Remove the platelet rich plasma by centrifuging once at 220-240 × g.
- Wash 2-3 times in PBS after the density gradient separation.
- After the final wash, resuspend the cells at a relatively high concentration in 1X BD IMag buffer, and proceed to step 3.

† Avoid nonspecific labeling by working quickly and adhering to recommended incubation times.

‡ The number and duration of washes (step 22) affects the results of the positive selection step. Purity and recovery are inversely related, such that:
- Fewer washes increases the recovery, but decreases the purity of $CD25^+$ $CD4^+$ cells.
- Similarly, longer time on the magnet increases the recovery, but decreases the purity of $CD25^+$ $CD4^+$ cells.
- Conversely, shorter time on the magnet increases the purity, but decreases the recovery of $CD25^+$ $CD4^+$ cells.

Ficoll-Paque is a trademark of Amersham Biosciences Limited.

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