Technical Data Sheet  
**Streptavidin Particles Plus - DM**

**Product Information**
- **Material Number:** 557812
- **Size:** 5.0 ml
- **Storage Buffer:** Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

**Description**
BD IMag™ Streptavidin Particles Plus - DM are magnetic nanoparticles that have streptavidin covalently conjugated to their surfaces. These particles are optimized for positive selection or depletion of leukocyte subpopulations using the BD IMagnet™.

**Preparation and Storage**
Antibody or streptavidin was conjugated to the magnetic particles under optimum conditions, and unconjugated antibody/streptavidin was removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.
Figure 2. Positive selection of human CD3-positive lymphocytes. Peripheral blood mononuclear cells (PBMC) were labeled with biotinylated anti-human CD3 mAb HIT3a (Cat. No. 555338) and BD™ IMag Streptavidin Particles - DM (at 20 µl per 10^7 total cells). After labeling, the cells were separated using the BD™ IMagnet, and the negative (CD3-) and positive (CD3+) fractions were collected. Please refer to the Positive Selection Flow Chart to identify the separated cell populations represented in this figure. For flow cytometric analysis, fresh PBMC (left panel), the negative fraction (middle panel), and the positive fraction (right panel) were stained with PE-conjugated anti-human CD3 mAb UCHT1 (Cat. No. 555333). The percent CD3+ cells in each sample is given. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system.

Application Notes

Recommended Assay Procedure:
A detailed Magnetic Labeling and Separation Protocol follows. In brief, cells are labeled with the biotinylated antibody, which recognizes the subpopulation of interest. After washing away excess antibody, BD IMag™ Streptavidin Particles Plus -DM are added to the cell suspension and bind the biotinylated antibody on the cells. The tube containing this labeled cell suspension is then placed within the magnetic field of the BD IMagnet™ (Cat. No. 552311). Positive selection or depletion is then performed. Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be drawn off (depleted or negative fraction). The tube is then removed from the magnetic field for resuspension of the positive fraction. The selections are repeated twice to increase the purity of the positive fraction and the yield of the depleted fraction. The magnetic separation steps are diagrammed in the accompanying Depletion and Positive Selection Flow Charts. The small size of the BD IMag™ particles allows the positive fraction to be further evaluated in downstream applications such as flow cytometry.

Optimal concentrations of BD IMag™ Streptavidin Particles Plus -DM for positive selection with some biotinylated monoclonal antibodies to human and mouse leukocyte antigens.

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Antibody Clone</th>
<th>Antibody Specificity</th>
<th>BD IMag Streptavidin Particle Concentration</th>
<th>Tissue Used</th>
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<tbody>
<tr>
<td>555411</td>
<td>HIB19</td>
<td>Human CD19</td>
<td>20 µl/10^7 total cells</td>
<td>PBMC</td>
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<tr>
<td>555338</td>
<td>HIT3a</td>
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<td>Mouse CD45R</td>
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<td>Mouse CD90.2</td>
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<td>53-6.7</td>
<td>Mouse CD8a</td>
<td>20 µl/10^7 total cells</td>
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<tr>
<td>551324</td>
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<tr>
<td>551328</td>
<td>M1/70</td>
<td>Mouse CD11b</td>
<td>20 µl/10^7 total cells</td>
<td>Bone Marrow</td>
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<td>551333</td>
<td>RB6-8C5</td>
<td>Mouse Ly-6G/Ly-6C</td>
<td>50 µl/10^7 total cells</td>
<td>Bone Marrow</td>
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</tbody>
</table>

For depletion of mouse hematopoietic cells bearing lineage-specific markers, we recommend the BD™ IMag Mouse Hematopoietic Stem Cell Enrichment Set - DM (Cat. No. 558451).

MAGNETIC LABELING AND SEPARATION PROTOCOL
1. Prepare buffers and place on ice.
   a. Cell-staining buffer: Phosphate Buffered Saline, 3% heat inactivated fetal calf serum, 0.1% sodium azide.
   b. 1X BD IMag buffer: Dilute BD™ IMag Buffer (10X) (Cat. No. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline, supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.*
2. Aseptically prepare a single-cell suspension from the lymphoid tissue of interest or prepare PBMC from anti-coagulated blood, preferably by density gradient centrifugation using the appropriate density Ficoll-Hypaque solution. Remove clumps of cells and/or debris by passing the
suspended cells through a 70-µm nylon cell strainer.

3. Count the cells, and resuspend them in cell-staining buffer at a concentration of 2 x 10^6 cells/ml.

4. Optional: If needed, add BD Mouse Fc Block™ purified anti-mouse CD16/CD32 mAb 2.4G2 (Cat. No. 553141/553142) or BD Rat Fc Block™ purified anti-rat CD32 mAb D34-485 (Cat. No. 550270/550271) at 0.25 µg/10^6 cells, and incubate on ice for 15 minutes.

5. Add the biotinylated antibody (or cocktail of biotinylated antibodies) at the appropriate concentration, and incubate on ice for 15 minutes.†

6. Wash the labeled cells with an excess volume of 1X BD IMag™ buffer, and carefully aspirate ALL the supernatant. For depletions, proceed with Step 7. For positive selections, proceed with Step 18.

**Depletions:**

7. Vortex the BD IMag™ Streptavidin Particles Plus - DM thoroughly, and add 50 µl of particles for every 1 x 10^7 total cells.

8. **MIX THOROUGHLY.** Refrigerate mouse or rat leukocytes for 30 minutes at 6°C -12°C. Incubate human PBMC at room temperature for 30 minutes.†

9. Bring the labeling volume up to 2 to 8 x 10^7 cells/ml with 1X BD IMag™ buffer or culture medium.*

10. Transfer the labeled cells to a 12 x 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 x 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.

11. With the tube on the BD IMagnet™ and using a glass Pasteur pipette, carefully aspirate the supernatant (depleted fraction) and place in a new tube.

12. Remove the positive-fraction tube from the BD IMagnet™, and add 1X BD IMag™ buffer (or medium) to the same volume as in Step 9. Resuspend the positive fraction well by pipetting up and down 10 to 15 times and place back on the BD IMagnet™ for 6 to 8 minutes.

13. x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.

14. Using a new Pasteur pipette, carefully aspirate the supernatant (wash fraction) and combine with the depleted fraction from Step 11 above.

15. Repeat Steps 12 and 13. The Combined Depleted Fraction contains cells with no bound antibodies or magnetic particles. These cells are ready for downstream applications, or they can be further enriched by proceeding to Step 16.

16. The positive-fraction cells remaining in the original tube can be resuspended in an appropriate buffer or culture medium for downstream applications, including flow cytometry.

17. To increase the purity of the Combined Depleted Fraction, place the tube on the BD IMagnet™ for another 6 to 8 minutes.

18. x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.

19. Carefully aspirate the supernatant and place in a new tube. This is the Final Depleted Fraction. The cells are ready to be processed for downstream applications.

**Positive Selections:**

18. Vortex the BD IMag™ Streptavidin Particles Plus - DM thoroughly, and add 10 to 50 µl of particles for every 1 x 10^7 total cells. The amount of particles to add will vary depending on how many cells one is targeting and the cell-surface density of the antigen. Please refer to the optimal concentration information on Page 2 for some common examples.

19. **MIX THOROUGHLY.** Refrigerate mouse or rat leukocytes for 30 minutes at 6°C -12°C. Incubate human PBMC at room temperature for 30 minutes.†

20. Bring the labeling volume up to 2 to 8 x 10^7 cells/ml with 1X BD IMag™ buffer.

21. Immediately place the tube onto the BD IMagnet™ and incubate for 6 to 8 minutes.

22. With the tube on the BD IMagnet™, carefully aspirate the supernatant. This supernatant is considered the Negative Fraction.

23. 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.

24. With the tube on the BD IMagnet™, carefully remove the supernatant (wash fraction).


26. After the final wash step, remove the tube from the BD IMagnet™. Resuspend the Positive Fraction in an appropriate buffer or culture medium, and proceed with desired downstream application(s), including flow cytometry.

**NOTES:**

*For depletion of mouse leukocytes, tissue culture medium usually results in a slight increase in viability and recovery, when compared to IMag buffer, without reducing cell purity. We recommend that researchers run a trial comparison of media to buffer to make sure that there are no adverse effects.

†Avoid non-specific labeling by working quickly and adhering to recommended incubation times.
Suggested Companion Products

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<thead>
<tr>
<th>Catalog Number</th>
<th>Name</th>
<th>Size</th>
<th>Clone</th>
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<tr>
<td>553141</td>
<td>Purified Rat Anti-Mouse CD16/CD32 (Mouse BD Fc Block™)</td>
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<td>Purified Mouse Anti-Rat CD32</td>
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<td>D34-485</td>
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<tr>
<td>552311</td>
<td>Cell Separation Magnet</td>
<td>each</td>
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<tr>
<td>558451</td>
<td>Mouse Hematopoietic Progenitor Cell Enrichment Set - DM</td>
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<td>PE Rat Anti-Mouse TER-119/Erythroid Cells</td>
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<td>555333</td>
<td>PE Mouse Anti-Human CD3</td>
<td>100 tests</td>
<td>UCHT1</td>
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Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/pharmingen/colors.
4. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology.
5. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
6. Source of all serum proteins is from USDA inspected abattoirs located in the United States.