TECHNICAL BULLETIN

Isolate Mouse CD45.1 or CD45.2 Positive Cells with EasySep™ Release Mouse Positive Selection Kits

CD45 is a type I transmembrane molecule found on the surface of all nucleated hematopoietic cells, and is an ideal target for mouse immune cell (i.e. leukocyte) isolation due to its high level of expression on these cells across different mouse strains.[1,2] CD45 exists in multiple isoforms as a result of alternative splicing of its mRNA.[3] In mice, CD45.1 and CD45.2 are common isoforms of the same functionally identical protein and are often used as markers to distinguish diverse genetic backgrounds, strains, and cell populations.[4] The alleles of the CD45.1 and CD45.2 isoforms differ only by 5 amino acids in their extracellular domains but share a common cytoplasmic domain.[4] This subtle change in the extracellular segment modifies the surface epitopes and allows specific epitope recognition of each CD45.1 and CD45.2 isoform by monoclonal antibodies.[4] CD45 is highly expressed, and its modification generally does not affect other cellular functions.

Studying mouse CD45.1 and CD45.2 cell populations is a valuable tool in stem cell and immunology research and offers a wide range of applications. For example, investigating CD45.1 and CD45.2 positive cells allows scientists to (1) detect chimerism in recipient mouse models; (2) design adoptive transfer experiments to test therapies and model diseases to analyze immune function and behavior; and (3) perform competitive studies where healthy cells compete with mutant cells to assess cell interactions and impact on immune and non-immune cells, among other applications.[1]

This technical bulletin introduces two first-of-their-kind protocols that provide a significant advantage over existing protocols for isolating mouse CD45.1 and CD45.2 positive cells—the ability to release bound magnetic particles following cell isolation. EasySep™ Release technology enables the efficient isolation of highly pure, particle-free cells, supporting more streamlined downstream analysis (e.g. single-cell sequencing, DNA/RNA analysis, etc.).

Why Use EasySep™ Release Technology to Isolate CD45.1 and CD45.2 Positive Cells?

- Remove bound magnetic particles from your cells of interest*
- Achieve purities of up to 95% in 44 - 60 minutes
- Easily isolate cells with a single pour
- Obtain functional cells using gentle EasySep™ technology
- Isolate cells from samples with varying starting frequencies

***IMPORTANT:** Following cell isolation with these EasySep™ Release kits, antibody complexes remain bound to the cell surface and may interact with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or other chemically related ligands.*

How Does EasySep™ Release Technology Work?

Magnetic cell separation, also known as immunomagnetic cell separation or magnetic cell sorting, involves targeting cells for selection or depletion using antibodies or ligands directed against specific cell surface antigens. Labeled cells are cross-linked to magnetic particles, also known as magnetic beads, that can be immobilized once an electromagnetic field is applied. Isolation of mouse CD45.1 and CD45.2 positive cells is typically performed using immunomagnetic positive selection. Therefore, once cell isolation is complete, magnetic particles usually remain bound to the surface of your cells of interest. However, the protocols described in this technical bulletin allow you to positively select your cells and then release bound magnetic particles using EasySep™ Release technology. Learn how to isolate CD45.1 and CD45.2 positive cells using the EasySep™ Release Mouse Biotin Positive Selection Kit (Catalog #17655) and EasySep™ Release Mouse PE Positive Selection Kit (Catalog #17656), respectively, and obtain particle-free, highly purified cells (Figures 1 and 2). Additionally, get details on how to isolate cells from mouse samples with varying starting frequencies, without compromising cell purity (Figures 1B and 2B).

Sample Preparation

Processing Mouse Spleen Samples

For details on how to prepare single-cell suspensions from mouse spleen samples, follow the steps below. The protocol can also be found on the product information sheet (PIS) of the EasySep™ Release Mouse Biotin Positive Selection Kit (Catalog #17655).

1. Disrupt the spleen in EasySep™ Buffer (Catalog #20144)
2. Remove aggregates and debris by passing the cell suspension through a 70 µm mesh nylon strainer (e.g. Catalog #27216)
3. Centrifuge the sample at 300 x *g* for 10 minutes
4. Resuspend the sample as described in Table 1 (step 1) and keep cells on ice until ready for use

IMPORTANT: *Using ammonium chloride is NOT recommended when preparing the cells for separation.*

You may also refer to our protocol and video on How to Prepare a Single-Cell Suspension from Mouse Spleen.

Processing Mouse Bone Marrow Samples

Follow the steps below to prepare single-cell suspensions from mouse bone marrow samples. An alternative protocol can also be found on the product information sheet (PIS) of the EasySep™ Release Mouse CD138 Positive Selection Kit (Catalog #100-0601).

1. Harvest the bones and snip one end of each to access the bone marrow
2. Transfer bones into a 0.5 mL tube with the cut side facing down (up to 4 bones per tube: 2 femurs and 2 tibiae)
3. Remove bone marrow cells from mouse femurs and tibiae by centrifugation as previously described by Heib et al. 2021:
    a. Use a needle to punch a hole in the bottom of a 0.5 mL tube
    b. Nest the 0.5 mL tube inside a 1.5 mL tube containing 100 µL EasySep™ Buffer (Catalog #20144)
    c. Add the femurs to the nested tube followed by the tibiae in order to prevent blocking the small hole in the bottom
    d. Centrifuge at 2500 x *g* for 1 minute at room temperature (RT)
4. Pool the cells using a micropipette
5. Pass the cells through a pre-wetted 70 µm mesh nylon strainer (e.g. Catalog #27216)
6. Centrifuge the single-cell suspension at 300 x *g* for 10 minutes at room temperature
7. Resuspend as described in Table 1 (step 1)

Isolating Mouse CD45.1 and CD45.2 Positive Cells From Other Tissues?

Optimization might be required. Please contact us at techsupport@stemcell.com for more information.

Protocols

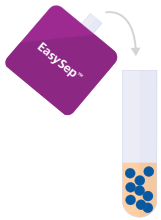
The protocols described in Table 1 will walk you through the isolation of CD45.1 (see table's middle column) and CD45.2 (see table's further right column) positive cells, using the EasySep™ Release Mouse Biotin Positive Selection Kit (Catalog #17655) and EasySep™ Release Mouse PE Positive Selection Kit (Catalog #17656), respectively.

IMPORTANT: *Both protocols were developed for use with the purple EasySep™ Magnet (Catalog #18000) and are optimized to isolate CD45.1 positive cells from mouse splenocytes and CD45.2 positive cells from mouse bone marrow cells.*

Table 1. Protocols to Isolate Mouse CD45.1 and/or CD45.2 Positive Cells Using EasySep™ Release Positive Selection Kits

Instructions	Isolate CD45.1 Positive Cells Using the EasySep™ Release Mouse Biotin Positive Selection Kit (Catalog #17655)	Isolate CD45.2 Positive Cells Using the EasySep™ Release Mouse PE Positive Selection Kit (Catalog #17656)
1. Prepare your sample at the indicated cell concentration within the recommended volume range and resuspend your cells in EasySep™ Buffer (Catalog #20144). ***Note:*** *Use 3% acetic acid with methylene blue (e.g. Catalog #07060) to count your nucleated cells with a hemocytometer (e.g. Catalog #100-1181).*	1×10^8 cells/mL Recommended volume: 0.25 - 0.5 mL	
Add your sample to the required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	
2. Add the EasySep™ Mouse FcR Blocker immediately before adding the selection antibody.	40 µL of EasySep™ Mouse FcR Blocker per mL of sample*	
3. Add the corresponding conjugated antibody to the sample. ***Note:*** *You may need to titrate the antibody for optimal purity and recovery. Contact us at* ***techsupport@stemcell.com*** *for more information.*	0.25 µg of biotin-conjugated Anti-Mouse CD45.1 Antibody, Clone A20 (Catalog #60117BT) per mL of sample	1.0 µg of PE-conjugated Anti-Mouse CD45.2 Antibody, Clone 104 (Catalog #100-1596) per mL of sample
Mix and incubate.	Room temperature for 5 minutes	
4. Add recommended medium** to top up the sample to the indicated volume.	Top up to 2.5 mL	
Centrifuge sample.	300 x *g* for 10 minutes	
5. Add the corresponding EasySep™ Release Positive Selection Cocktail to the sample. ***Note:*** *(1) DO NOT vortex cocktail. (2) You may need to titrate the cocktail for optimal purity and recovery. Contact us at* ***techsupport@stemcell.com*** *for more information.*	50 µL of EasySep™ Release Biotin Positive Selection Cocktail per mL of sample	50 µL of EasySep™ Release PE Positive Selection Cocktail per mL of sample
Mix and incubate.	Room temperature for 3 minutes	
6. Add EasySep™ Releasable RapidSpheres™. ***Note:*** *Vortex RapidSpheres™ for 30 seconds before use, particles should appear evenly dispersed.*	100 µL per mL of sample	
Mix and incubate.	Room temperature for 3 minutes	

Instructions (CONTINUED)	Isolate CD45.1 Positive Cells Using the EasySep™ Release Mouse Biotin Positive Selection Kit (Catalog #17655)	Isolate CD45.2 Positive Cells Using the EasySep™ Release Mouse PE Positive Selection Kit (Catalog #17656)
7. Perform immunomagnetic isolation as follows: Add recommended medium** to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	
Place the tube (without lid) into the purple EasySep™ Magnet (Catalog #18000).	Room temperature for 5 minutes 	
Pick up the magnet, and in one continuous motion***, invert the magnet and tube, pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	
8. Repeat steps as indicated.	Repeat step 7 as follows: (A) If your starting frequency is ≤ 30% total: Repeat 3 more times for a total of 4 x 5-minute separations Or (B) If your starting frequency is > 30% total: Repeat 2 more times for a total of 3 x 5-minute separations	
9. Add EasySep™ Release Buffer (1X) to resuspend and top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. ***Note:*** *EasySep™ Release Buffer (1X) must be prepared on the day of use. Dilute concentrated buffer 1 in 40 with recommended medium***.*	Top up to 2.5 mL	
Incubate.	Room temperature for 3 minutes	
10. Place the tube (without lid) into the EasySep™ Magnet (Catalog #18000) and incubate.	Room temperature for 5 minutes	

Instructions (CONTINUED)	Isolate CD45.1 Positive Cells Using the EasySep™ Release Mouse Biotin Positive Selection Kit (Catalog #17655)	Isolate CD45.2 Positive Cells Using the EasySep™ Release Mouse PE Positive Selection Kit (Catalog #17656)
11. Pick up the magnet, and in one continuous motion***, invert the magnet and tube, pouring the enriched cell suspension into a new tube.	Keep supernatant (collected in the new tube): Isolated, particle-free cells are ready for use! 	

**Please note: Figures 2A and 2B were generated using 40 and 20 µL of EasySep™ Mouse FcR blocker per mL of sample, respectively. Although cell purity is similar within this range, we recommend adding 40 µL of Mouse FcR blocker per mL of sample.*

***EasySep™ Buffer (Catalog #20144), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.*

****Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain around the mouth of the tube. For visual examples and demonstration videos, check out our tech tip, "How to Collect Supernatant from EasySep™ Magnets".*

Supporting Data: Obtain Particle-Free, Highly Purified Cells

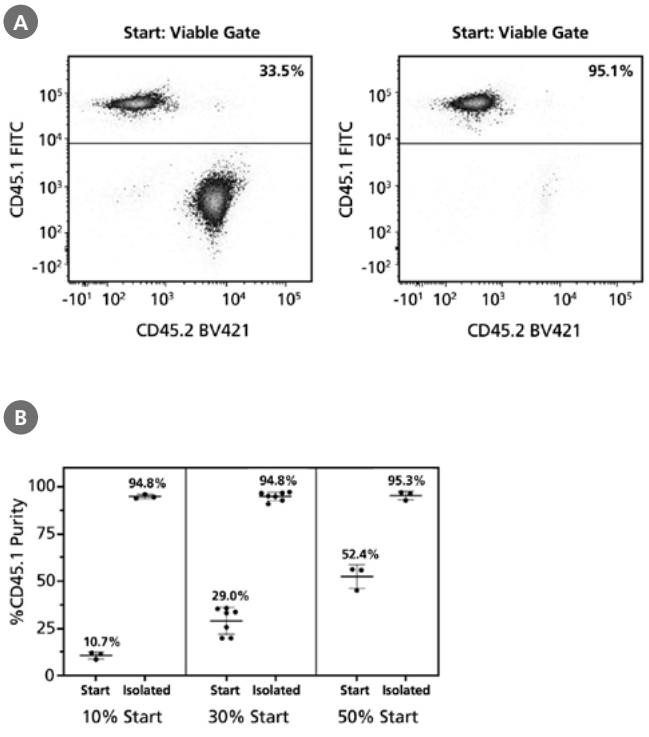


Figure 1. Using the EasySep™ Release Mouse Biotin Positive Selection Kit to Isolate CD45.1 Positive Cells Results in High Cell Purity

Cell isolation was performed using the purple EasySep™ Magnet (Catalog #18000), the EasySep™ Release Mouse Biotin Positive Selection Kit (Catalog #17655), and biotin-conjugated, Anti-Mouse CD45.1 Antibody, Clone A20 (Catalog #60117BT), as described under the "Protocols" section of this technical bulletin (Table 1).

(A) Typical Cell Isolation Profile Yields High Cell Purity
Starting with a 30% CD45.1 positive mouse splenocyte spike-in model, mixed with CD45.2 positive splenocytes, the CD45.1 positive cell content of the isolated fraction is 94.8% ± 2.3% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 33.5% and 95.1%, respectively.

(B) Cell Purity Remains High Across Different Cell Starting Frequencies
CD45.1 positive mouse splenocytes were mixed with CD45.2 positive mouse splenocytes at frequencies of approximately 10%, 30%, or 50%. The figure shows the start frequency of CD45.1 positive cells and the post-release purity of CD45.1 after isolation. For starting frequencies of ~10%, 30%, and 50%, the purities of the isolated fractions are 94.8% ± 1.1%, 94.8% ± 2.3%, and 95.3% ± 2.2% (mean ± SD), respectively. Each data point is an independent experiment.

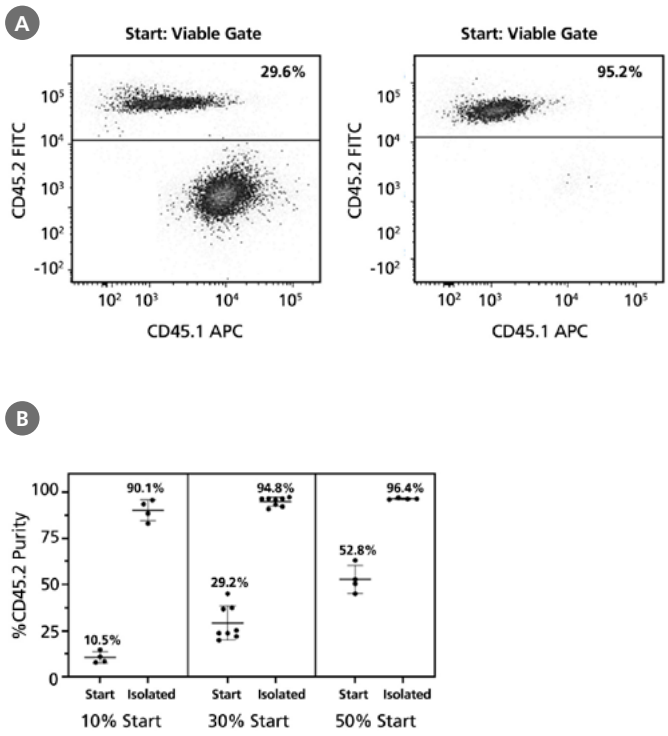


Figure 2. Using the EasySep™ Release Mouse PE Positive Selection Kit to Isolate CD45.2 Positive Cells Results in High Cell Purity

Cell isolation was performed using the purple EasySep™ Magnet (Catalog #18000), the EasySep™ Release Mouse PE Positive Selection Kit (Catalog #17656), and a PE-conjugated, Anti-Mouse CD45.2 Antibody, Clone 104 (Catalog #100-1596), as described under the "Protocols" section of this technical bulletin (Table 1).

(A) Typical Cell Isolation Profile Yields High Cell Purity
Starting with a 30% CD45.2 positive mouse bone marrow cell spike-in model, mixed with CD45.1 positive bone marrow cells, CD45.2 positive cell content of the isolated fraction is 94.8% ± 2.4% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 29.6% and 95.2%, respectively.
Note: Cells were first labeled with a PE-CD45.2 antibody for selection, then isolated according to the protocol described in Table 1. Isolated cells were stained with FITC-CD45.2 to determine purity and exclude any cells with non-specific binding of PE-CD45.2.

(B) Cell Purity Remains High Across Different Cell Starting Frequencies
CD45.2 positive mouse bone marrow cells were mixed with CD45.1 positive mouse bone marrow cells at frequencies of approximately 10%, 30%, or 50%. The figure shows the start frequency of CD45.2 positive cells and the post-release purity of CD45.2 after isolation. For starting frequencies of ~10%, 30%, and 50%, the purities of the isolated fractions are 90.1% ± 1.9%, 94.8% ± 2.4%, and 96.4% ± 0.5% (mean ± SD), respectively. Each data point is an independent experiment.

Product Information

Product	Unit Size	Catalog #
EasySep™ Release Mouse Biotin Positive Selection Kit	1 Kit	17655
EasySep™ Release Mouse PE Positive Selection Kit	1 Kit	17656
Anti-Mouse CD45.1 Antibody, Clone A20, Biotin	500 µg	60117BT
Anti-Mouse CD45.2 Antibody, Clone 104, PE (Phycoerythrin)	100 µg	100-1596
EasySep™ Magnet	1 Unit	18000
EasySep™ Buffer	1 L	20144
3% Acetic Acid with Methylene Blue	100 mL	07060
Hausser Scientific™ Bright-Line Hemocytometer	1 Unit	100-1181
Falcon® Round-Bottom Polystyrene Tubes, 5 mL	500 Tubes	38007
Reversible Strainers	70 µm, Small	27216

Looking for Something Else?

Find our complete listing of negative and positive selection kits and our most current EasySep™ mouse T cell isolation, culture, and characterization products at **www.stemcell.com/Mouse-Tcell**

References

1. Mercier FE et al. (2016) Single targeted exon mutation creates a true congenic mouse for competitive hematopoietic stem cell transplantation: the C57BL/6-CD45.1(STEM) mouse. Stem Cell Rep 6(6): 985–92.
2. Hermiston ML et al. (2009) CD45, CD148, and Lyp/Pep: critical phosphatases regulating Src family kinase signaling networks in immune cells. Immunol Rev. 228(1): 288–311.
3. Penninger JM et al. (2001) CD45: new jobs for an old acquaintance. Nat Immunol. 2(5): 389–96.
4. Jang Y et al. (2018) Cutting Edge: Check Your Mice-A Point Mutation in the Ncr1 Locus Identified in CD45.1 Congenic Mice with Consequences in Mouse Susceptibility to Infection. J Immunol. 200(6):1982–7.
5. Heib T et al. (2021) Isolation of murine bone marrow by centrifugation or flushing for the analysis of hematopoietic cells - a comparative study. 32(5): 601–7.

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TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713 • INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

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