# EasySep™ Mouse TIL (CD45) Positive Selection Kit

**For processing 1 x 10^9 cells**

Catalog #100-0350

Positive Selection

Document #10000008193 | Version 01

STEMCELL™ TECHNOLOGIES

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## Description

Isolate highly purified CD45+ tumor infiltrating leukocytes (TILs) by immunomagnetic positive selection. This kit has been optimized for use on single-cell suspensions of solid mouse tumors, including tumors induced by implantation of 4T1, B16-F10, and CT26.WT cell lines into syngeneic mice. Due to the heterogeneity of mouse tumors, this kit may require optimization.

- Fast, easy-to-use and column-free
- Optimized for tumors and tissue samples with a low CD45 start frequency
- Flexible protocols for higher purity or recovery

This kit targets CD45+ cells for positive selection with antibodies recognizing the CD45 surface marker. Desired cells are labeled with antibodies and magnetic particles and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications, such as flow cytometry, culture, and cell-based experiments.

## Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|---|---|---|---|---|
| EasySep™ Mouse TIL (CD45) Positive Selection Cocktail Component A | 300-0145 | 1 x 0.25 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS with 0.1% BSA. |
| EasySep™ Mouse TIL (CD45) Positive Selection Cocktail Component B | 300-0146 | 1 x 0.25 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS with 5% HPCD. |
| EasySep™ Mouse TIL (CD45) Positive Selection Cocktail Component C | 300-0147 | 1 x 1.5 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A solution that enhances the performance of the isolation cocktail. |
| EasySep™ Dextran RapidSpheres™ 50100 | 50100 | 2 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles in water. |

BSA - bovine serum albumin; HPCD - 2-hydroxypropyl-β-cyclodextrin; PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

## Sample Preparation

For automated and standardized tissue processing, see STEMprep™ Tissue Dissociator (Catalog #100-2112) at www.stemcell.com/stemprep. For manual processing, follow the steps below.

The protocol below is an example for generating a single-cell suspension from solid tumors from mouse models of breast cancer (4T1), melanoma (B16-F10), and colon cancer (CT26.WT), but it may be applicable to a variety of other tissues.

TUMOR TISSUE

The following instructions are for processing ≤ 1 g of tumor tissue. For > 1 g of tumor tissue, adjust volumes accordingly.

1. Prepare 5 mL of tumor digestion medium by combining the following:
   - 500 µL Collagenase/Hyaluronidase Solution (Catalog #07912)
   - 750 µL DNase I Solution (1 mg/mL; Catalog #07900)
   - 3.75 mL RPMI 1640 Medium (Catalog #36750)

   Mix thoroughly and warm to room temperature (15 - 25°C).
2. Harvest the tumor tissue into a dish (e.g. Catalog #27100).
3. Mince the tumor tissue into small pieces (≤ 2 mm) using a razor blade, scalpel, or scissors.
4. Transfer the minced tumor tissue to a 14 mL round-bottom tube (e.g. Catalog #38008) containing tumor digestion medium (prepared in step 1).
5. Incubate at 37°C for 25 minutes on a shaking platform.
6. Place a 70 µm nylon mesh strainer (e.g. Catalog #27260) on a 50 mL conical tube (e.g. Catalog #38010) and rinse with recommended medium. Transfer the digested tumor tissue into the strainer. Using the rubber end of a syringe plunger, push digested tumor tissue through the strainer. Rinse the strainer with recommended medium, then top up the tube to 50 mL with recommended medium.
7. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
8. Add 10 mL of Ammonium Chloride Solution (Catalog #07800) to the cell pellet. Incubate at room temperature for 5 minutes.
9. Top up to 50 mL with recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant. Resuspend cells at 5 x 10^7 cells/mL in recommended medium.

   NOTE: For higher TIL purity, resuspend cells at 5 - 10 x 10^7 cells/mL; for higher TIL recovery, resuspend cells at 1 - 2.5 x 10^7 cells/mL in recommended medium.

## Recommended Medium

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

# Directions for Use – Manual EasySep™ Protocols

See page 2 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

**Table 1. EasySep™ Mouse TIL (CD45) Positive Selection Kit Protocol**

| | | EASYSEP™ MAGNETS | |
|---|---|---|---|
| STEP | INSTRUCTIONS | **EasySep™ (Catalog #18000)** | **"The Big Easy" (Catalog #18001)** |
| 1 | **Prepare sample at the indicated cell concentration within the volume range.** | 5 x 10^7 cells/mL<br>0.1 - 1.5 mL<br>NOTE: For higher purity, 5 - 10 x 10^7 cells/mL is recommended.<br>For higher recovery, 1 - 2.5 x 10^7 cells/mL is recommended. | 5 x 10^7 cells/mL<br>0.5 - 8 mL<br>NOTE: For higher purity, 5 - 10 x 10^7 cells/mL is recommended.<br>For higher recovery, 1 - 2.5 x 10^7 cells/mL is recommended. |
| | **Add sample to required tube.** | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Catalog #38007) | 14 mL (17 x 95 mm) polystyrene round-bottom tube<br>(e.g. Catalog #38008) |
| 2 | **Prepare Selection Cocktail in a tube. For each 1 mL of sample, prepare 100 µL of cocktail (12.5 µL of Component A + 12.5 µL of Component B + 75 µL of Component C).** | Prepare Selection Cocktail as indicated.<br>NOTE: Selection Cocktail must be prepared fresh before each use. | Prepare Selection Cocktail as indicated.<br>NOTE: Selection Cocktail must be prepared fresh before each use. |
| 3 | **Add Selection Cocktail to sample.**<br>**NOTE: Do not vortex cocktail.** | 100 µL/mL of sample | 100 µL/mL of sample |
| | **Mix and incubate.** | RT for 5 minutes | RT for 5 minutes |
| 4 | **Vortex RapidSpheres™.**<br>**NOTE: Particles should appear evenly dispersed.** | 30 seconds | 30 seconds |
| 5 | **Add RapidSpheres™ to sample.** | 50 µL/mL of sample | 50 µL/mL of sample |
| | **Mix and incubate.** | RT for 3 minutes | RT for 3 minutes |
| 6 | **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 | • Top up to 5 mL for samples < 3 mL<br>• Top up to 10 mL for samples ≥ 3 mL |
| | **Place the tube (without lid) into the magnet and incubate.** | RT for 5 minutes | RT for 5 minutes |
| 7 | **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 | Discard supernatant |
| 8 | **Repeat steps as indicated.** | Steps 6 and 7, three more times<br>(total of 4 x 5-minute separations) | Steps 6 and 7, three more times<br>(total of 4 x 5-minute separations) |
| 9 | **Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.** | Isolated cells are ready for use | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)

* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

**Table 2. EasySep™ Mouse TIL (CD45) Positive Selection Kit Protocol**

| | | EASYSEP™ MAGNETS | |
|---|---|---|---|
| | | EasyEights™ (Catalog #18103) | |
| STEP | INSTRUCTIONS | 5 mL tube | 14 mL tube |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10^7 cells/mL<br>0.1 - 1.5 mL<br>NOTE: For higher purity, 5 - 10 x 10^7 cells/mL is recommended.<br>For higher recovery, 1 - 2.5 x 10^7 cells/mL is recommended. | 5 x 10^7 cells/mL<br>0.5 - 8 mL<br>NOTE: For higher purity, 5 - 10 x 10^7 cells/mL is recommended.<br>For higher recovery, 1 - 2.5 x 10^7 cells/mL is recommended. |
| | Add sample to required tube. | 5 mL (12 x 75 mm) polystyrene round-bottom tube<br>(e.g. Catalog #38007) | 14 mL (17 x 95 mm) polystyrene round-bottom tube<br>(e.g. Catalog #38008) |
| 2 | Prepare Selection Cocktail in a tube. For each 1 mL of sample, prepare 100 µL of cocktail (12.5 µL of Component A + 12.5 µL of Component B + 75 µL of Component C). | Prepare Selection Cocktail as indicated.<br>NOTE: Selection Cocktail must be prepared fresh before each use. | Prepare Selection Cocktail as indicated.<br>NOTE: Selection Cocktail must be prepared fresh before each use. |
| 3 | Add Selection Cocktail to sample.<br>NOTE: Do not vortex cocktail. | 100 µL/mL of sample | 100 µL/mL of sample |
| | Mix and incubate. | RT for 3 minutes | RT for 3 minutes |
| 4 | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds |
| 5 | Add RapidSpheres™ to sample. | 100 µL/mL of sample | 100 µL/mL of sample |
| | Mix and incubate. | RT for 3 minutes | RT for 3 minutes |
| 6 | 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 | • Top up to 5 mL for samples < 3 mL<br>• Top up to 10 mL for samples ≥ 3 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 10 minutes | RT for 10 minutes |
| 7 | Carefully pipette** (do not pour) off the supernatant. Remove the tube, containing the isolated cells, from the magnet. | Discard supernatant | Discard supernatant |
| 8 | 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 | • Top up to 5 mL for samples < 3 mL<br>• Top up to 10 mL for samples ≥ 3 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes | RT for 5 minutes |
| 9 | Carefully pipette** (do not pour) off the supernatant. Remove the tube, containing the isolated cells, from the magnet. | Discard supernatant | Discard supernatant |
| 10 | Repeat steps as indicated. | Steps 8 and 9, two more times<br>(total of 1 x 10-minute and 3 x 5-minute separations) | Steps 8 and 9, two more times<br>(total of 1 x 10-minute and 3 x 5-minute separations) |
| 11 | Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube. | Isolated cells are ready for use | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).

## Notes and Tips

ASSESSING PURITY

For purity assessment by flow cytometry, use one of the following antibody clones:

- Anti-Mouse CD45 Antibody, Clone 30-F11 (Catalog #60030; may be partially blocked) for all mouse strains, or
- Anti-Mouse CD45.2 Antibody, Clone 104 (Catalog #60118) for CD45.2- or Ly5.2-bearing mouse strains (e.g. A, AKR, BALB/c, CBA/Ca, CBA/J, C3H/He, C57BL, C57BR, C57L, C58, DBA/1, DBA/2, NZB, SWR, and 129), or
- Anti-Mouse CD45.1 Antibody, Clone A20 (Catalog #60117) for CD45.1- or Ly5.1-bearing mouse strains (e.g. RIII, SJL/J, STS/A, and DA)

## Data

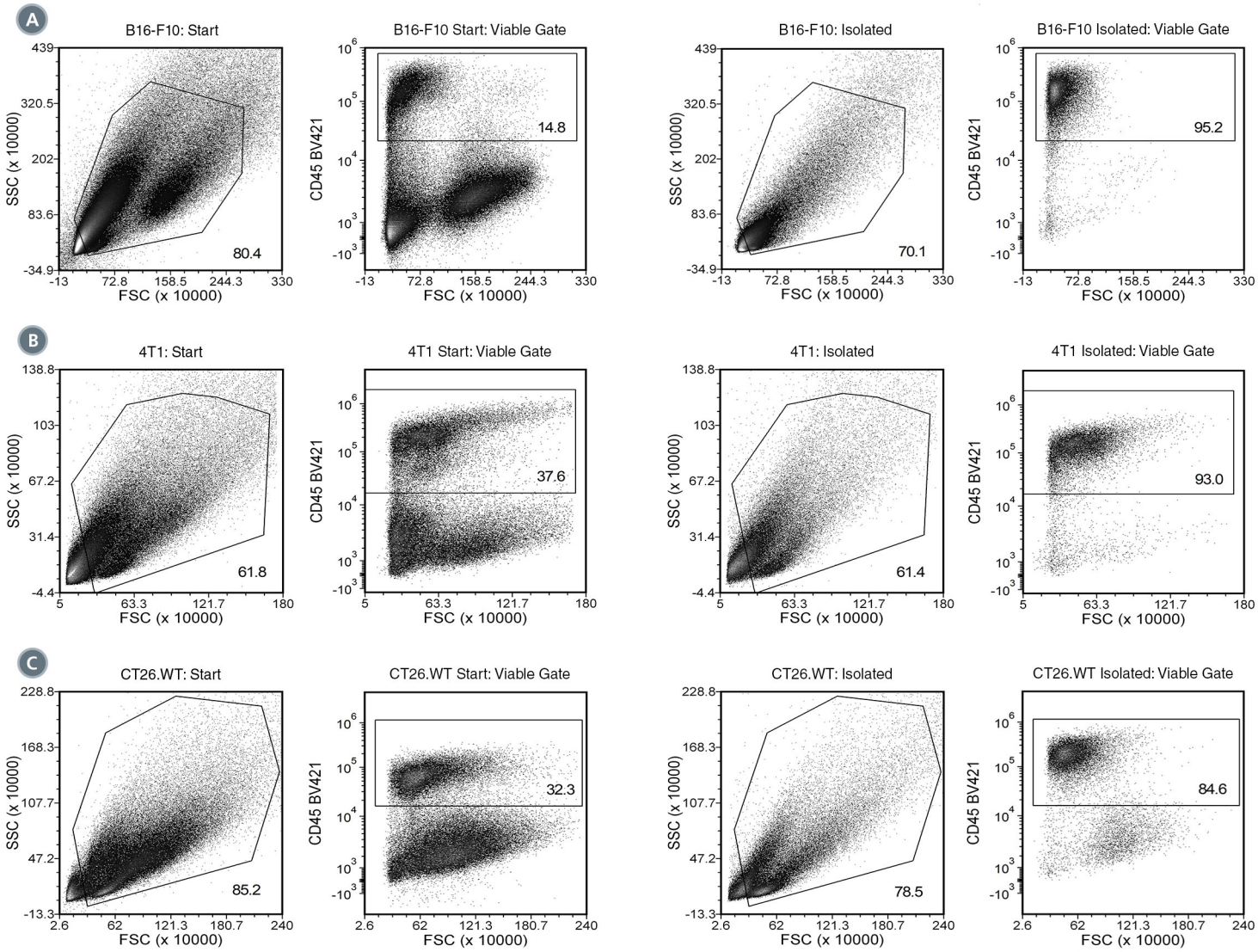


**Figure 1. Isolation of CD45+ Tumor Infiltrating Leukocytes (TILs) from Mouse Tumors**

Tumors were induced by B16-F10, 4T1, or CT26.WT cell lines and dissociated into single-cell suspensions. CD45+ TILs were isolated from single-cell suspensions at various start concentrations using the purple EasySep™ Magnet.
(A) Starting with a B16-F10 tumor single-cell suspension at 1 x 10^8 cells/mL, the purities of the start and final isolated fractions are 14.8% and 95.2%, respectively.
(B) Starting with a 4T1 tumor single-cell suspension at 4 x 10^7 cells/mL, the purities of the start and final isolated fractions are 37.6% and 93.0%, respectively.
(C) Starting with a CT26.WT tumor single-cell suspension at 2.5 x 10^7 cells/mL, the purities of the start and final isolated fractions are 32.3% and 84.6%, respectively.

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