EasySep™ Mouse TIL (CD45) Positive Selection Kit

For processing 1 x 10^9 cells

Catalog #100-0350

Positive Selection

Document #10000008193 | Version 00

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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

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 1 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 0.1 - 1.5 mL NOTE: For higher purity, 5 - 10 x 10^7 cells/mL is recommended. For higher recovery, 1 - 2.5 x 10^7 cells/mL is recommended.	5 x 10^7 cells/mL 0.5 - 8 mL NOTE: For higher purity, 5 - 10 x 10^7 cells/mL is recommended. 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 (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (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. NOTE: Selection Cocktail must be prepared fresh before each use.	Prepare Selection Cocktail as indicated. NOTE: Selection Cocktail must be prepared fresh before each use.
3	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
4	Vortex RapidSpheres™. 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 • 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 (total of 4 x 5-minute separations)	Steps 6 and 7, three more times (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 0.1 - 1.5 mL NOTE: For higher purity, 5 - 10 x 10^7 cells/mL is recommended. For higher recovery, 1 - 2.5 x 10^7 cells/mL is recommended.	5 x 10^7 cells/mL 0.5 - 8 mL NOTE: For higher purity, 5 - 10 x 10^7 cells/mL is recommended. 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 (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (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. NOTE: Selection Cocktail must be prepared fresh before each use.	Prepare Selection Cocktail as indicated. NOTE: Selection Cocktail must be prepared fresh before each use.
3	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
4	Vortex RapidSpheres™. 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 • 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 • 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 (total of 1 x 10-minute and 3 x 5-minute separations)	Steps 8 and 9, two more times (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]).

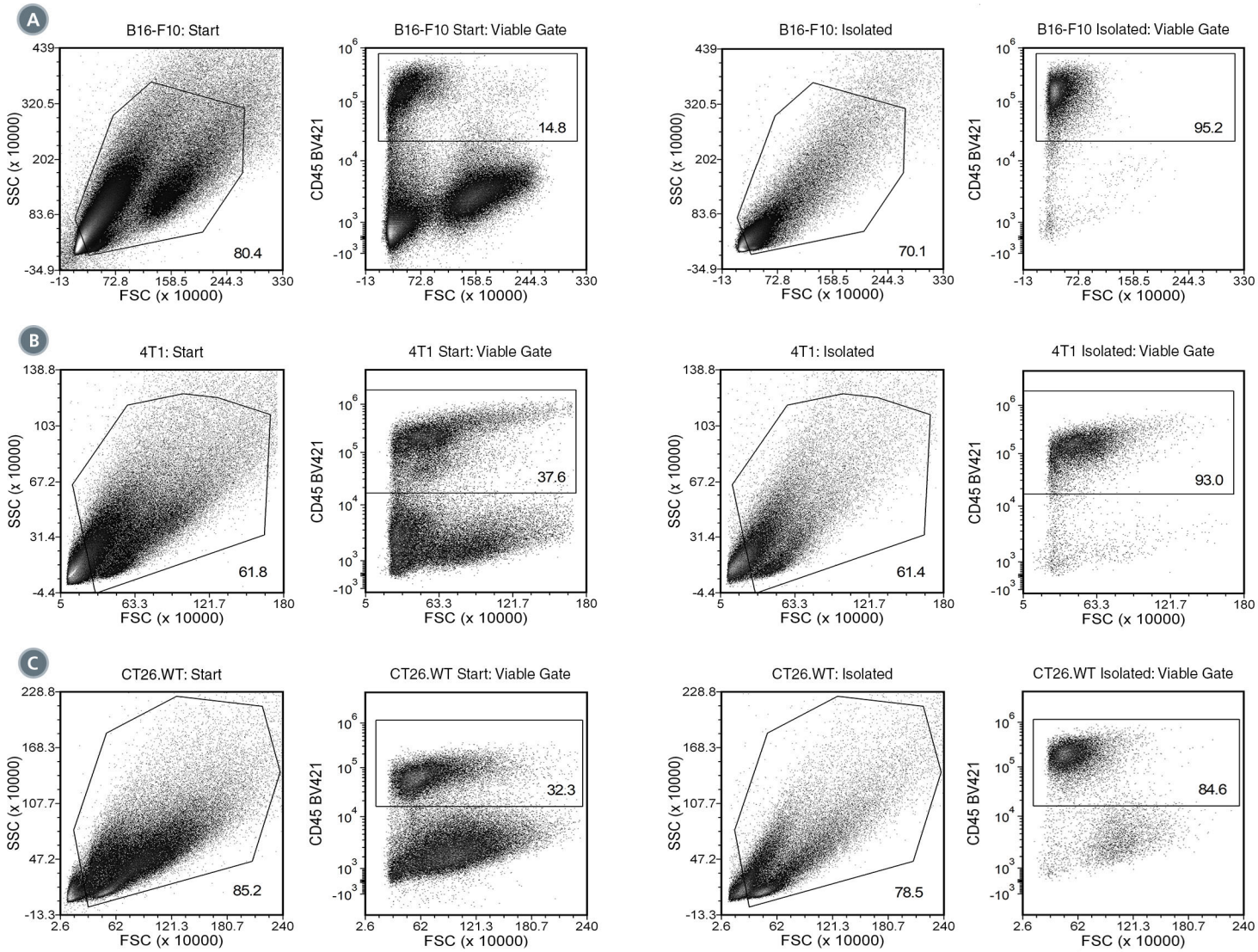
Notes and Tips

ASSESSING PURITY

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

- Anti-Mouse CD45, Clone 30-F11 (Catalog #60030; may be partially blocked) for all mouse strains, or
- Anti-Mouse CD45.2, 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, Clone A20 (Catalog #60117) for CD45.1- or Ly5.1-bearing mouse strains (e.g. RIII, SJL/J, STS/A, and DA)

Data



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.