EasySep™ Release Human CD45 Positive Selection Kit

For processing 1 x 10^9 cells

Catalog #100-0105
Catalog #100-0108 RoboSep™
Catalog #100-0107 (for Humanized Mice)
Catalog #100-0109 RoboSep™ (for Humanized Mice)

Positive Selection

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Description

Isolate human CD45+ leukocytes from single-cell suspensions of primary human tissues and tumors (Catalog #100-0105), or tissues and tumor xenografts from humanized mice (Catalog #100-0107).

- Highly purified human CD45+ leukocytes and tumor-infiltrating leukocytes (TILs) in less than 45 minutes
- No-wash removal of EasySep™ Releasable RapidSpheres™

This kit targets CD45+ cells for positive selection with antibodies recognizing the CD45 surface marker and EasySep™ Releasable RapidSpheres™. 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. Bound magnetic particles are then removed from the EasySep™-isolated CD45+ cells, which are immediately available for downstream applications such as flow cytometry, culture, and DNA/RNA extraction.

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

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Release Human CD45 Positive Selection Cocktail	300-0046	1 x 0.5 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™ Releasable RapidSpheres™ 50201	50201	1 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.
EasySep™ Release Buffer (Concentrate)	20165	6 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A buffer for release of Releasable RapidSpheres™ from cells following positive selection.
EasySep™ Mouse FcR Blocker*	18731	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A monoclonal antibody in PBS with 0.1% BSA and < 0.1% sodium azide.

BSA - bovine serum albumin; PBS - phosphate-buffered saline

* Supplied only with EasySep™ Release Human CD45 Positive Selection Kit for Humanized Mice (Catalog #100-0107) and RoboSep™ Release Human CD45 Positive Selection Kit for Humanized Mice (Catalog #100-0109).

Components may be shipped at room temperature (15 - 25°C) and should be stored according to their storage conditions upon receipt.

Robosep™ Release Human CD45 Positive Selection Kit (Catalog #100-0108) and Robosep™ Release Human CD45 Positive Selection Kit for Humanized Mice (Catalog #100-0109) are supplied with EasySep™ EasyTube™-14 (Component #20128) for optimal performance. EasySep™ EasyTube™-14 is not required when performing a manual separation.

Sample Preparation

Prepare a single-cell suspension prior to performing cell isolation using a dissociation protocol optimized for viability and yield. The protocol below is an example for generating a single-cell suspension from a human breast cancer tumor xenograft (MDA-MB-231), but it may be applicable to a variety of other tissues.

HUMAN TUMOR XENOGRAFTS

1. Prepare tumor digestion medium.

   The following example is for preparing 5 mL of tumor digestion medium for ≤ 1 g of minced tumor tissue. For > 1 g of minced tumor tissue, adjust volumes accordingly.

   - 500 µL of Collagenase/Hyaluronidase Solution (Catalog #07912)
   - 750 µL of DNase Solution (1 mg/mL; Catalog #07900)
   - 3.75 mL of RPMI 1640 Medium (Catalog #36750)

   Mix thoroughly.
2. Harvest the tumor tissue into a 35 mm culture dish (e.g. Catalog #27100).
3. Mince the tumor tissue into small pieces (≤ 2 mm) using a razor blade or scalpel.
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).

   NOTE: Use 5 mL of tumor digestion medium for ≤ 1 g of minced tumor tissue. For > 1 g of minced tumor tissue, adjust volume accordingly.
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. Resuspend cells at 1 x 10^8 cells/mL in recommended medium.

NOTE: For samples with high red blood cell (RBC) content, lysis using Ammonium Chloride Solution (Catalog #07800) is recommended.

Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% fetal bovine serum (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™ Release Human CD45 Positive Selection Kit or EasySep™ Release Human CD45 Positive Selection Kit for Humanized Mice Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Dilute Release Buffer (Concentrate) to prepare release buffer (1X).	Dilute 1 in 40 with recommended medium. Note: Release buffer (1X) must be prepared on the day of use. Refer to steps 10 and 13 for required volume.	Dilute 1 in 40 with recommended medium. Note: Release buffer (1X) must be prepared on the day of use. Refer to steps 10 and 13 for required volume.
2	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.1 - 2 mL	1 x 10^8 cells/mL 0.25 - 8 mL
	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)
3	If isolating cells from human samples (Catalog #100-0105), proceed directly to step 4. OR If isolating cells from humanized mice (Catalog #100-0107), add FcR blocker to sample.	10 µL/mL of sample	10 µL/mL of sample
4	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add Releasable RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
7	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 ≤ 4 mL • Top up to 10 mL for samples > 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
8	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
Continue to step 9, next page		Continue to step 9, next page	Continue to step 9, next page

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
9	**Repeat steps as indicated.**	Steps 7 and 8, three more times (total of 4 x 5-minute separations)	Steps 7 and 8, three more times (total of 4 x 5-minute separations)
10	**Add release buffer (1X) 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 ≤ 4 mL • Top up to 10 mL for samples > 4 mL
	Incubate.	RT for 3 minutes	RT for 3 minutes
11	**Place the tube (without lid) into the magnet and incubate.**	RT for 5 minutes	RT for 5 minutes
12	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pour the supernatant into a new tube and set aside.**	Use a new 14 mL tube NOTE: Isolated cells (in the new tube) will be combined with the poured-off fraction in step 15	Use a new 14 mL tube NOTE: Isolated cells (in the new tube) will be combined with the poured-off fraction in step 15
13	**Remove the tube from the magnet and add release buffer (1X) 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 ≤ 4 mL • Top up to 10 mL for samples > 4 mL
14	**Place the tube (without lid) into the magnet and incubate.**	RT for 5 minutes	RT for 5 minutes
15	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension.**	Combine with poured-off fraction from step 12 Isolated cells are ready for use	• Use a new 14 mL tube for start samples ≤ 4 mL • Use a new 50 mL tube for start samples > 4 mL Combine with poured-off fraction from step 12 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™ Release Human CD45 Positive Selection Kit or EasySep™ Release Human CD45 Positive Selection Kit for Humanized Mice Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube 
1	Dilute Release Buffer (Concentrate) to prepare release buffer (1X).	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to steps 10 and 13 for required volume.	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to steps 10 and 13 for required volume.	Dilute 1 in 40 with recommended medium. NOTE: Release buffer (1X) must be prepared on the day of use. Refer to steps 10 and 13 for required volume.
2	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.05 - 0.2 mL	1 x 10^8 cells/mL 0.1 - 2 mL	1 x 10^8 cells/mL 0.25 - 8 mL
	Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).	Round-bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018)	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)
3	If isolating cells from human samples (Catalog #100-0105), proceed directly to step 4. OR If isolating cells from humanized mice (Catalog #100-0107), add FcR blocker to sample.	10 µL/mL of sample	10 µL/mL of sample	10 µL/mL of sample
4	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
5	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
6	Add Releasable RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
7	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 4 mL • Top up to 10 mL for samples > 4 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
8	Carefully pipette* (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant	Discard supernatant
Continue to step 9, next page		Continue to step 9, next page	Continue to step 9, next page	Continue to step 9, next page

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS (CONTINUED)	 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube 
9	Repeat steps as indicated.	Steps 7 and 8, three more times (total of 4 x 5-minute separations)	Steps 7 and 8, three more times (total of 4 x 5-minute separations)	Steps 7 and 8, three more times (total of 4 x 5-minute separations)
10	Add release buffer (1X) to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 4 mL • Top up to 10 mL for samples > 4 mL
	Incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
11	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
12	Carefully pipette* (do not pour) off the supernatant into a new tube or plate and set aside.	Use a new plate NOTE: Isolated cells (in the new plate) will be combined with the pipetted-off fraction in step 15	Use a new 14 mL tube NOTE: Isolated cells (in the new tube) will be combined with the pipetted-off fraction in step 15	Use a new 14 mL tube NOTE: Isolated cells (in the new tube) will be combined with the pipetted-off fraction in step 15
13	Remove the tube or plate from the magnet and add release buffer (1X) to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 4 mL • Top up to 10 mL for samples > 4 mL
14	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
15	Carefully pipette* (do not pour) the isolated cell suspension off.	Combine with pipetted-off fraction from step 12 Isolated cells are ready for use	Combine with pipetted-off fraction from step 12 Isolated cells are ready for use	• Use a new 14 mL tube for start samples ≤ 4 mL • Use a new 50 mL tube for start samples > 4 mL Combine with pipetted-off fraction from step 12 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]).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 2 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Release Human CD45 Positive Selection Kit or RoboSep™ Release Human CD45 Positive Selection Kit for Humanized Mice Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.25 - 8 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	If isolating cells from human samples (Catalog #100-0105), proceed directly to step 3. OR If isolating cells from humanized mice (Catalog #100-0107), add FcR blocker to sample.	10 µL/mL of sample
3	Select protocol.	• Release Human CD45 Positive Selection 100-0105 - small volume (< 4 mL) • Release Human CD45 Positive Selection 100-0105 - large volume (4 - 8 mL) • Release Humanized Mouse CD45 Positive Selection 100-0107 - small volume (< 4 mL) • Release Humanized Mouse CD45 Positive Selection 100-0107 - large volume (4 - 8 mL)
4	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Load the carousel.	Follow on-screen prompts NOTE: When prompted to load a separation tube, place EasySep™ EasyTube™-14 into the magnet.
	Start the protocol.	Press the green “Run” button
6	Unload the carousel when the run is complete.	Isolated cells are ready for use

Notes and Tips

EASYSEP™ RELEASE BUFFER

EasySep™ Release Buffer (Concentrate) is supplied as a 40X concentrate; release buffer (1X) must be prepared on the day of use. To prepare release buffer (1X), dilute an appropriate volume 1 in 40 with recommended medium. Refer to steps 10 and 13 of Table 1 and Table 2 for required volume.

OPTIMIZING RECOVERY

For a high recovery protocol, steps 7 - 9 of Table 1 and Table 2 may be reduced to a total of 2 x 5-minute separations.

LOW STARTING CELL NUMBERS

For low starting cell numbers (e.g. fewer than 1 x 10^7 cells for the purple EasySep™ magnet in Table 1), resuspend cells in the minimum recommended volume in step 2 before proceeding with the protocol.

ASSESSING PURITY

For purity assessment of human leukocytes (CD45+) by flow cytometry, use the following fluorochrome-conjugated antibody clone:

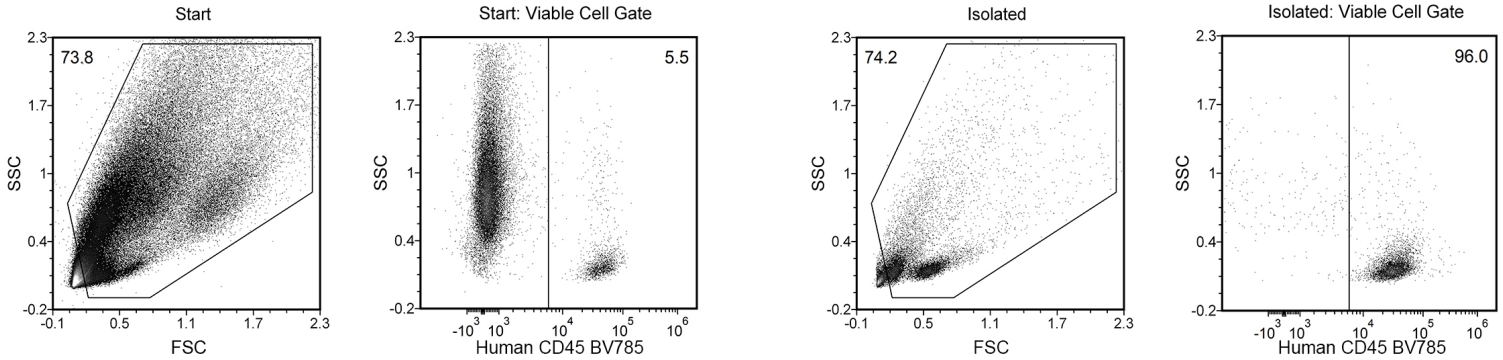
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018; may be partially blocked)

NOTE: Use of a viability dye is strongly recommended.

NOTE: To exclude debris for purity assessment, use of the cell-permeant nuclear dye DRAQ5™ is recommended.

NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For purity assessment with Brilliant Violet™ antibody conjugates, use of BD Horizon Brilliant™ Stain Buffer is recommended to reduce non-specific interactions. For more information, refer to the manufacturer’s instructions or contact us at techsupport@stemcell.com.

Data



Starting with a single-cell suspension of a human breast cancer tumor xenograft (MDA-MB-231) sample from an NRG-3GS humanized mouse, the CD45+ TIL purities of the start and final isolated fractions are 5.5% and 96.0%, respectively.

NOTE: Cell debris and dead cells were excluded from the analysis based on DRAQ5™ and DAPI fluorescence.