EasySep™

Positive Selection

Catalog #17869

EasySep™ Human Central and Effector Memory CD8+ T Cell Isolation Kit

For processing 1 x 10^9 cells

STEMCELL™ TECHNOLOGIES

Scientists Helping Scientists™ | WWW.STEMCELL.COM

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

Document #DX21929 | Version 1_1_0

Description

Isolate highly purified central (CD3+CD8+CD45RO+CD62L+) and effector (CD3+CD8+CD45RO+CD62L-) memory CD8+ T cells from fresh peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples by immunomagnetic positive selection.

- Easy-to-use and column-free
- Up to 93% purity
- No-wash removal of EasySep™ Releasable RapidSpheres™

First, CD62L+ cells are isolated by column-free immunomagnetic positive selection using antibody complexes and EasySep™ Releasable RapidSpheres™. Then, bound magnetic particles are removed from the EasySep™-isolated CD62L+ cells, and unwanted CD45RA+ and non-CD8+ T cells are targeted for depletion using antibody complexes and EasySep™ Dextran RapidSpheres™. The effector memory CD8+ T cells are isolated from the CD62L- fraction using antibody complexes and EasySep™ Dextran RapidSpheres™. Following cell isolation with this EasySep™ Release kit, 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.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Release Human CD62L Positive Selection Cocktail	17756C	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 0.1% BSA.
EasySep™ Human Memory CD8+ T Cell Isolation Cocktail	17869C	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.
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™ Dextran RapidSpheres™ 50102	50102	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	20145	1 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.

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

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

Sample Preparation

For available fresh samples, see www.stemcell.com/primarycells.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube. After preparation, resuspend cells at 1 x 10^8 cells/mL in recommended medium.

* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

LEUKAPHERESIS (LEUKO PAK)

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). If isolating effector memory CD8+ T cells, lyse red blood cells (RBCs) with Ammonium Chloride Solution (Catalog #07800). If platelet removal is desired, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

NOTE: CD62L expression may be lost due to shedding. For optimal performance, use samples collected within the last 24 hours. Do not use frozen samples.

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™ Human Central Memory CD8+ T Cell Isolation 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.	1 x 10^8 cells/mL 0.5 - 2 mL	1 x 10^8 cells/mL 1 - 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)
2	Add CD62L Positive 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
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add Releasable RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
5	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 1.25 mL for samples < 1 mL • Top up to 2.5 mL for samples ≥ 1 mL	• Top up to 2.5 mL for samples < 2 mL • Top up to 5 mL for samples ≥ 2 - 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
6	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.	Set aside supernatant for isolating effector memory CD8+ T cells (Table 3) if desired.	Set aside supernatant for isolating effector memory CD8+ T cells (Table 3) if desired.
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 1.25 mL for samples < 1 mL • Top up to 2.5 mL for samples ≥ 1 mL	• Top up to 2.5 mL for samples < 2 mL • Top up to 5 mL for samples ≥ 2 - 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

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.

		EASYSEP™ MAGNETS	
STEP	**INSTRUCTIONS (CONTINUED)**	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
9	**Repeat steps as indicated.**	Steps 7 and 8 (total of 3 x 5-minute separations)	Steps 7 and 8 (total of 3 x 5-minute separations)
10	**Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. Be sure to collect cells off the sides of the tube.**	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)
11	**Add Release Buffer to sample and mix.**	100 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	100 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step
12	**Add Memory CD8+ T Cell Isolation Cocktail to sample.**	50 µL/mL of resuspended sample	50 µL/mL of resuspended sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
13	**Vortex Dextran RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
	Add Dextran RapidSpheres™ to sample and mix.	40 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	40 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step
14	**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 1.25 mL for samples < 1 mL • Top up to 2.5 mL for samples ≥ 1 mL	• Top up to 2.5 mL for samples < 2 mL • Top up to 5 mL for samples ≥ 2 - 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
15	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.**	Use a new 5 mL tube	Use a new 14 mL tube
16	**Remove the tube from the magnet. Place the new tube containing the enriched cells (without lid) into the magnet and incubate for a second separation.**	RT for 5 minutes	RT for 5 minutes
17	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new 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.
§ The resuspended sample volume should be used to determine the volume of reagents added in subsequent steps.

Table 2. EasySep™ Human Central Memory CD8+ T Cell Isolation Protocol

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002) 
		5 mL tube 	14 mL tube 	
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.5 - 2 mL	1 x 10^8 cells/mL 1 - 8 mL	1 x 10^8 cells/mL 10 - 40 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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add CD62L Positive Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
3	Vortex Releasable RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
4	Add Releasable RapidSpheres™ to sample.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
5	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 1.25 mL for samples < 1 mL • Top up to 2.5 mL for samples ≥ 1 mL	• Top up to 2.5 mL for samples < 2 mL • Top up to 5 mL for samples ≥ 2 - 4 mL • Top up to 10 mL for samples > 4 mL	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) off the supernatant.	Set aside supernatant for isolating effector memory CD8+ T cells (Table 4) if desired.	Set aside supernatant for isolating effector memory CD8+ T cells (Table 4) if desired.	Set aside supernatant for isolating effector memory CD8+ T cells (Table 4) if desired.
7	Remove the tube from the magnet and 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 1.25 mL for samples < 1 mL • Top up to 2.5 mL for samples ≥ 1 mL	• Top up to 2.5 mL for samples < 2 mL • Top up to 5 mL for samples ≥ 2 - 4 mL • Top up to 10 mL for samples > 4 mL	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
8	Carefully pipette** (do not pour) off the supernatant.	Discard supernatant	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8 (total of 3 x 10-minute separations)	Steps 7 and 8 (total of 3 x 10-minute separations)	Steps 7 and 8 (total of 3 x 10-minute separations)
Continue to step 10 next page		Continue to step 10, next page	Continue to step 10, next page	Continue to step 10, next page

RT - room temperature (15 - 25°C)

** Collect the entire supernatant, all at once, into a single pipette (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]).

STEP	INSTRUCTIONS (CONTINUED)	EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002) 
		5 mL tube	14 mL tube	
10	Remove the tube from the magnet and add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times. Be sure to collect cells off the sides of the tube.	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)	Half of the original starting sample volume§ (i.e. half of the volume used in step 1)
11	Add Release Buffer to sample and mix.	100 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	100 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	100 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step
12	Add Memory CD8+ T Cell Isolation Cocktail to sample.	50 µL/mL of resuspended sample	50 µL/mL of resuspended sample	50 µL/mL of resuspended sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
13	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
	Add Dextran RapidSpheres™ to sample and mix.	80 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	80 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	80 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step
14	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 1.25 mL for samples < 1 mL • Top up to 2.5 mL for samples ≥ 1 mL	• Top up to 2.5 mL for samples < 2 mL • Top up to 5 mL for samples ≥ 2 - 4 mL • Top up to 10 mL for samples > 4 mL	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
15	Carefully pipette** (do not pour) off the supernatant.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
16	Remove the tube from the magnet. Place the new tube containing the enriched cells (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
17	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use

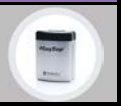
** Collect the entire supernatant, all at once, into a single pipette (for EasyEights™ 5 mL tube use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).
§ The resuspended sample volume should be used to determine the volume of reagents added in subsequent steps.

Table 3. Human Effector Memory CD8+ T Cell Enrichment Protocol

		EASYSEP™ MAGNETS	
STEP	**INSTRUCTIONS**	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Ensure cells are placed in the required tube.	Supernatant from Table 1, step 6 must be in a 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	Supernatant from Table 1, step 6 must be in a 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
	Centrifuge cells and resuspend in recommended medium to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Discard supernatant Half of the original starting sample volume§ (i.e. half of the volume used in Table 1, step 1)	Discard supernatant Half of the original starting sample volume§ (i.e. half of the volume used in Table 1, step 1)
2	Add Memory CD8+ T Cell Isolation Cocktail to sample.	50 µL/mL of resuspended sample	50 µL/mL of resuspended sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
	Add Dextran RapidSpheres™ to sample and mix.	40 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	40 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step
4	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 1.25 mL for samples < 1 mL • Top up to 2.5 mL for samples ≥ 1 mL	• Top up to 2.5 mL for samples < 2 mL • Top up to 5 mL for samples ≥ 2 - 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
5	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
6	Remove the tube from the magnet. Place the new tube containing the enriched cells (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
7	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use

* 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.
§ The resuspended sample volume should be used to determine the volume of reagents added in subsequent steps.

Table 4. Human Effector Memory CD8+ T Cell Enrichment Protocol

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		Easy 50 (Catalog #18002) 
STEP	INSTRUCTIONS (CONTINUED)	 5 mL tube	14 mL tube 	
1	Ensure cells are placed in the required tube.	Supernatant from Table 2, step 6 must be in a 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	Supernatant from Table 2, step 6 must be in a 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	Supernatant from Table 2, step 6 must be in a 50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
	Centrifuge cells and resuspend in recommended medium to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Discard supernatant Half of the original starting sample volume§ (i.e. half of the volume used in Table 2, step 1)	Discard supernatant Half of the original starting sample volume§ (i.e. half of the volume used in Table 2, step 1)	Discard supernatant Half of the original starting sample volume§ (i.e. half of the volume used in Table 2, step 1)
2	Add Memory CD8+ T Cell Isolation Cocktail to sample.	50 µL/mL of resuspended sample	50 µL/mL of resuspended sample	50 µL/mL of resuspended sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
3	Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
	Add Dextran RapidSpheres™ to sample and mix.	80 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	80 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step	80 µL/mL of resuspended sample NO INCUBATION, proceed immediately to next step
4	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 1.25 mL for samples < 1 mL • Top up to 2.5 mL for samples ≥ 1 mL	• Top up to 2.5 mL for samples < 2 mL • Top up to 5 mL for samples ≥ 2 - 4 mL • Top up to 10 mL for samples > 4 mL	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
5	Carefully pipette** (do not pour) off the supernatant.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
6	Remove the tube from the magnet. Place the new tube containing the enriched cells (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
7	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use

** Collect the entire supernatant, all at once, into a single pipette (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]).
§ The resuspended sample volume should be used to determine the volume of reagents added in subsequent steps.

Notes and Tips

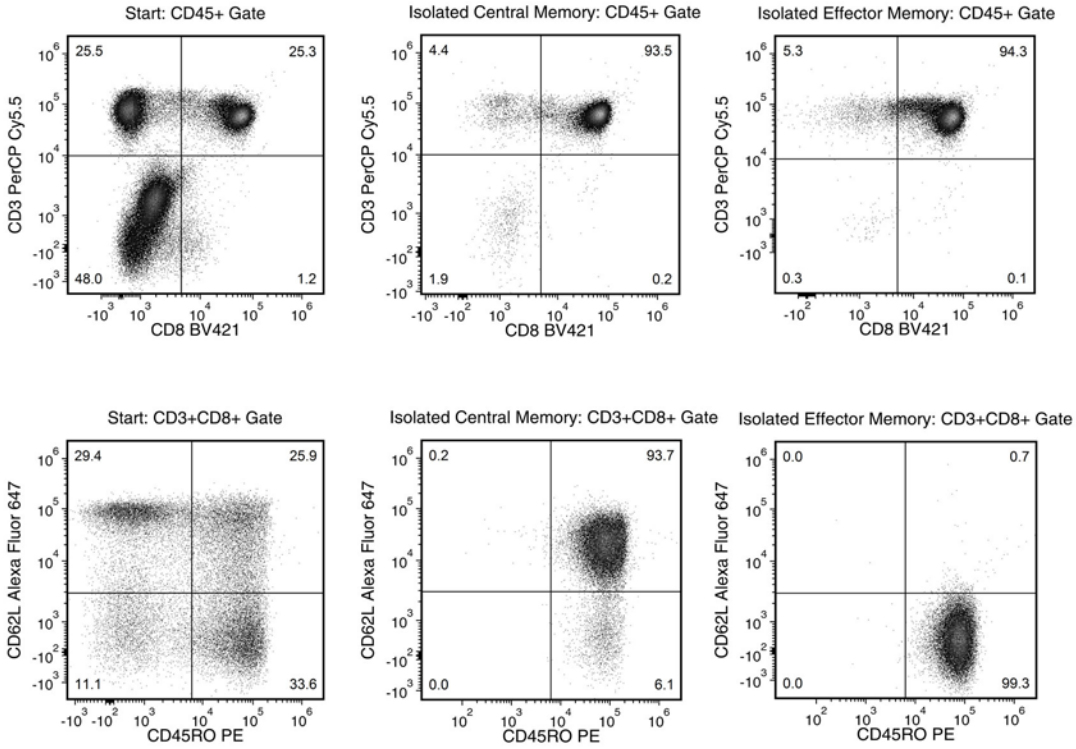
ASSESSING PURITY

For purity assessment of central memory (CD3+CD8+CD45RO+CD62L+) or effector memory (CD3+CD8+CD45RO+CD62L-) CD8+ T cells by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone SK7 (Catalog #60127), and
- Anti-human CD8 antibody, clone RPA-T8, and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018), and
- Anti-human CD45RA antibody, clone HI100 (optional), and
- Anti-Human CD45RO Antibody, Clone UCHL1 (Catalog #60097), and
- Anti-human CD62L antibody, clone DREG56 (partially blocked)

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 fresh PBMCs, the central memory CD8+ T cell content (CD3+CD8+CD45RO+CD62L+) of the isolated fraction is typically 86.8 ± 8.4% (mean ± SD using the purple EasySep™ magnet). The effector memory content (CD3+CD8+CD45RO+CD62L-) of the isolated fraction is typically 88.7 ± 6.5% (mean ± SD).

NOTE: RBCs were removed from the start sample by lysis prior to flow cytometry.