EasySep™

Negative Selection

Catalog #19582

EasySep™ Non-Human Primate CD4+ T Cell Isolation Kit

For processing 1 x 10^9 cells

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Description

Isolate untouched and highly purified CD4+ T cells from fresh or previously frozen non-human primate peripheral blood mononuclear cells (PBMCs) in as little as 25 minutes by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 85% purity with high recovery
- Untouched, viable cells

This kit targets non-CD4+ T cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications such as flow cytometry, cell culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Non-Human Primate CD4+ T Cell Isolation Cocktail	19582C	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 0.09% sodium azide.
EasySep™ D Magnetic Particles	19250	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 TBS.

PBS - phosphate-buffered saline; TBS - Tris-buffered saline

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

Sample Preparation

This kit has been verified for use with rhesus and cynomolgus macaques.

PERIPHERAL BLOOD

For peripheral whole blood from rhesus macaques, prepare a PBMC suspension by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For peripheral whole blood from cynomolgus macaques, dilute the density gradient medium to 90% using D-PBS (Without Ca++ and Mg++; Catalog #37350).

NOTE: For higher recovery, 14 mL polystyrene round-bottom tubes (e.g. Catalog #38008) are recommended for density gradient centrifugation, particularly for smaller volumes of peripheral blood.

For samples > 24 hours old, it may be necessary to lyse the red blood cells (RBCs) using Ammonium Chloride Solution (Catalog #07800) prior to cell isolation.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (e.g. Catalog #27250) for optimal results.

After preparation, resuspend cells at 5 x 10^7 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), 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™ Non-Human Primate CD4+ T Cell Isolation 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 0.5 - 2 mL	5 x 10^7 cells/mL 0.5 - 6 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 Isolation Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	**Vortex EasySep™ D Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add Magnetic Particles to sample.**	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 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 2.5 mL	• Top up to 5 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate. **NOTE: Purity may be improved by increasing incubation time to 10 minutes; however, this may reduce recovery.**	RT for 5 minutes	RT for 5 minutes
6	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the cell suspension into a new tube.**	Use a new 5 mL tube	Use a new 14 mL tube
7	**Place the new tube from step 6 (without lid) into the magnet and incubate for a second separation.**	RT for 5 minutes	RT for 5 minutes
8	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the cell suspension into a new tube.**	Use a new 5 mL tube Isolated cells are ready for use	Use a new 14 mL tube 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.

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Non-Human Primate CD4+ T Cell Isolation Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	**Prepare sample at the indicated cell concentration within the volume range.**	5 x 10^7 cells/mL 0.5 - 6 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Select protocol.**	Non-Human Primate CD4+ T Cell Isolation 19582
3	**Vortex EasySep™ D Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
4	**Load the carousel.**	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	**Unload the carousel when the run is complete. Remove the tube containing the isolated cells.**	Isolated cells are ready for use

Notes and Tips

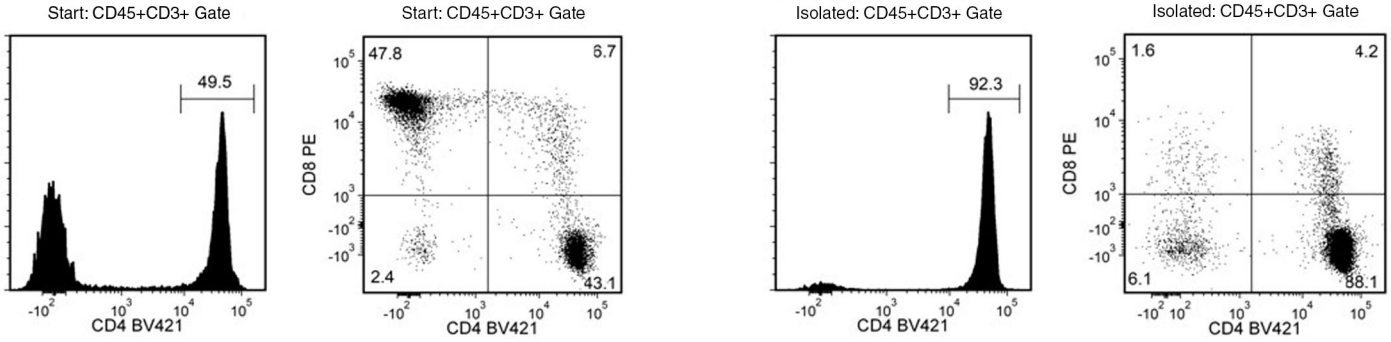
ASSESSING PURITY

NOTE: Due to the presence of residual RBCs, use of a CD45 antibody is strongly recommended.
NOTE: Use of a cell viability dye is strongly recommended.

For purity assessment of non-human primate CD4+ T cells (CD3+CD4+) by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-human CD3 antibody, clone SP34.2,
- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016),
- Anti-Human CD8 Antibody, Clone SK1 (Catalog #60022), and
- Anti-human CD45 antibody, clone D058-1283

Data



In the above example, starting with rhesus macaque PBMCs, the CD4+ T cell content (within the CD45+CD3+ gate) of start and final isolated fractions are 49.5% and 92.3%, respectively.