Immunomagnetic Purification of Human Central and Effector Memory T Cell Subsets in 45 Minutes

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Introduction

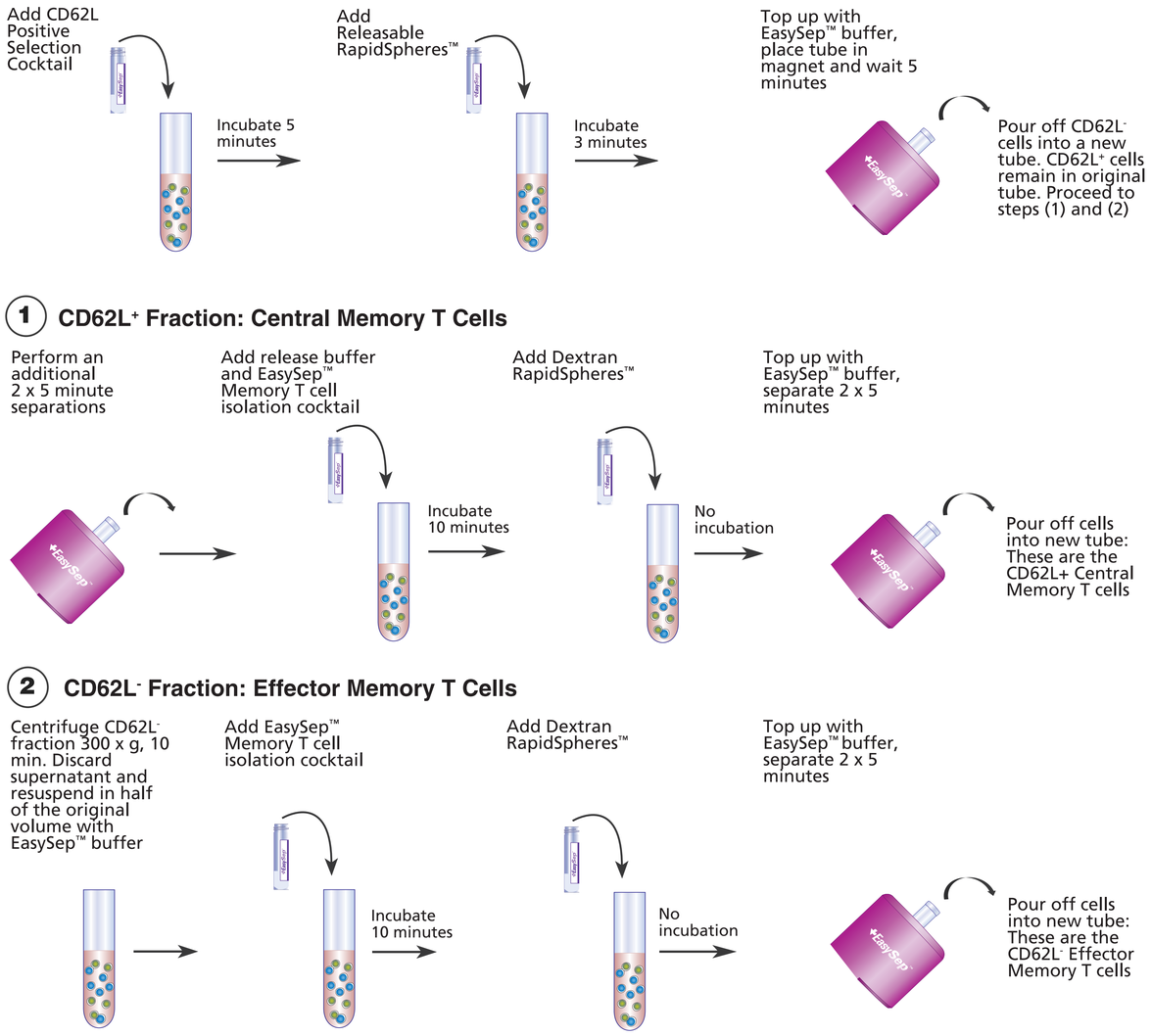
Simple methods for the isolation of memory T cell subsets with complex phenotypes are invaluable for continued advancements in the fields of infectious disease and immunotherapy. Central and effector memory T cells differ in their proliferative potential, effector function, and homing capacity. Their distinct trafficking is governed by the expression of lymph node homing receptors CD62L and CCR7. The expression of CD62L on central but not on effector memory T cells forms the basis for isolations of the two subsets from a single sample. We describe the isolation of particle-free central and effector memory CD4+ or CD8+ T cells by a novel sequential immunomagnetic cell separation strategy.

Starting from fresh leukapheresis samples or PBMCs, cells expressing CD62L are positively selected using our EasySep™ Release technology and an EasySep™ magnet. Particles are then released from the CD62L positive fraction and non-T cells, CD4+ or CD8+ T cells, and naïve T cells are targeted for depletion by antibody complexes crosslinked to our EasySep™ Dextran RapidSpheres™. To isolate effector memory CD4+ or CD8+ T cells, the retained CD62L negative fraction is depleted of non-effector memory cells by a second negative selection step.

The EasySep™ Central Memory and Effector Memory CD4+ and CD8+ T cell kits offer outstanding speed and ease of use for the purification of functional memory T cell subsets ready for downstream assays.

Fast and Easy Method

Starting From Fresh Leukapheresis Samples or PBMCs



Results

	EasySep™ Human Central Memory and Effector Memory CD4+ T Cell Isolation Kit			EasySep™ Human Central Memory and Effector Memory CD8+ T Cell Isolation Kit		
	Purity (%)	Recovery (%)	n	Purity (%)	Recovery (%)	n
Central Memory	92.8 ± 3.6	46.4 ± 13.9	23	88.0 ± 6.0	27.5 ± 8.7	16
Effector Memory	93.8 ± 3.2	59.4 ± 16.0	17	90.9 ± 4.9	27.6 ± 9.0	15

Table 1. Average purity and recovery ± standard deviation of central and effector memory CD4+ or CD8+ T cells using the new EasySep™ Human Central and Effector Memory CD4+ T cell Isolation Kit or EasySep™ Human Central and Effector Memory CD8+ T cell Isolation Kit. Cells were isolated using a purple or silver EasySep™ magnet.

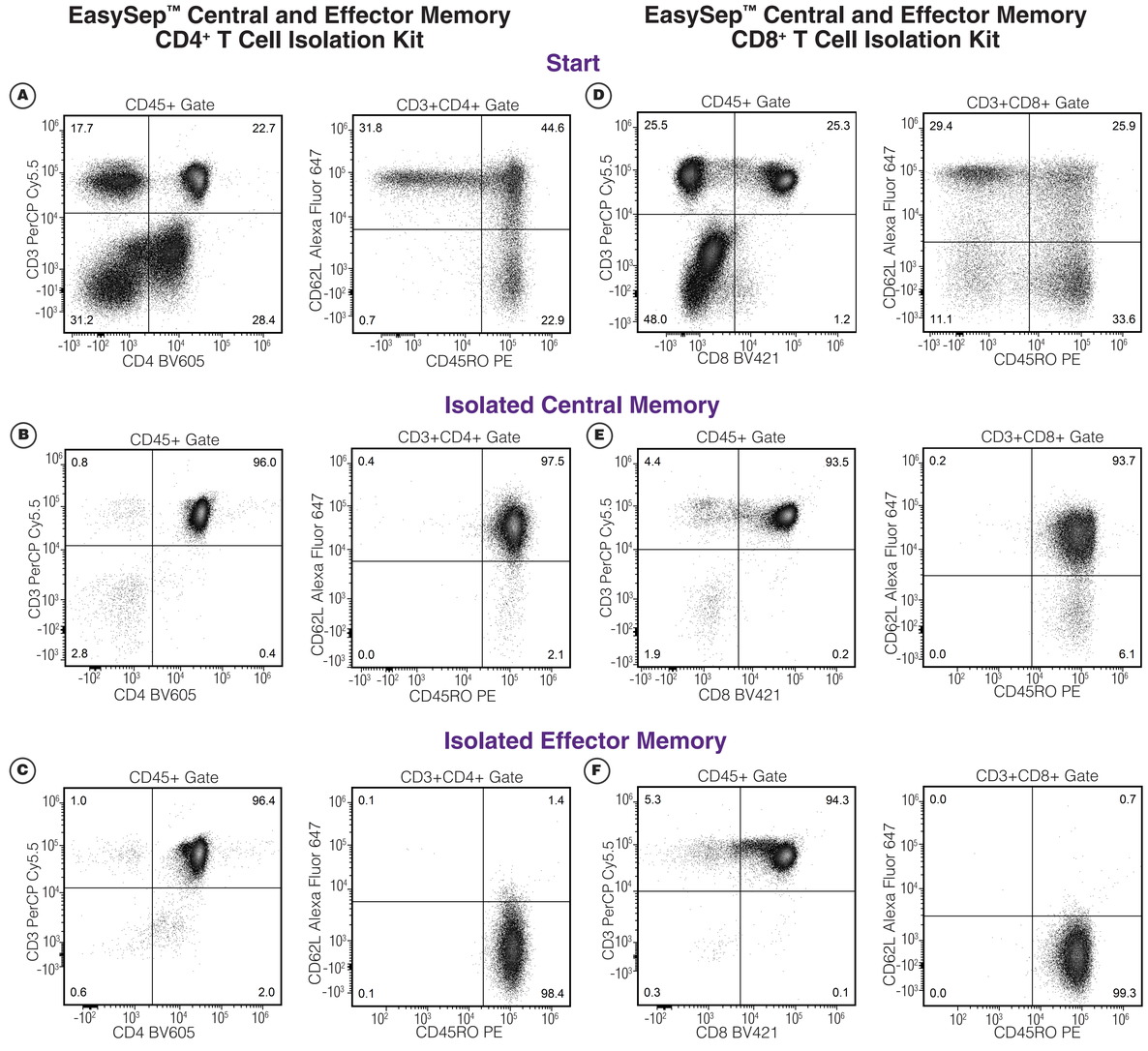


Figure 1. Typical EasySep™ Human Central Memory and Effector Memory T Cell Isolation Kit Purities. (A) Start populations in fresh PMBCs and final isolated populations for **(B)** central memory CD4+ T cells, and **(C)** effector memory CD4+ T cells. **(D)** Start populations in fresh PMBCs and final isolated populations for **(E)** central memory CD8+ T cells, and **(F)** effector memory CD8+ T cells**.**

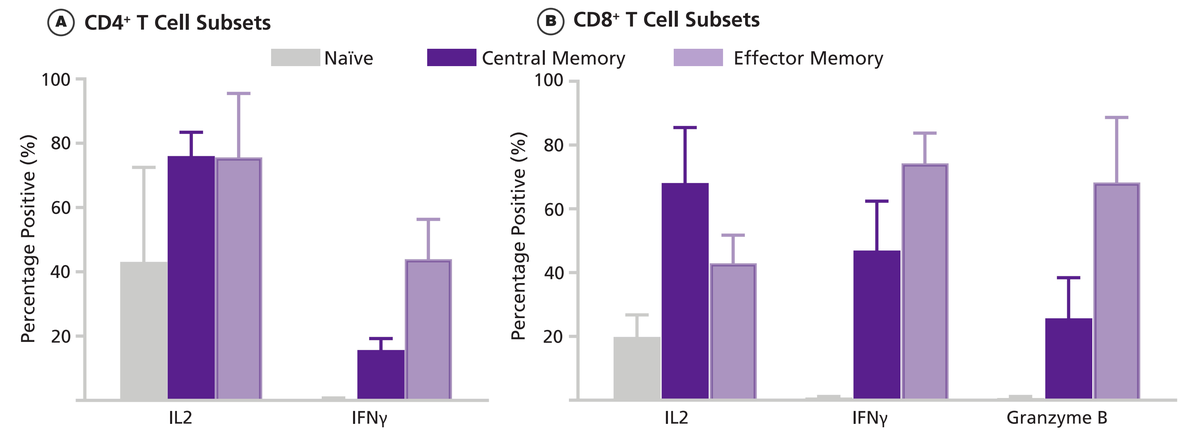


Figure 2. Isolated T cell subsets produce IL-2, IFNγ, and granzyme B at expected levels upon stimulation. (A) Cells isolated using the EasySep™ Human Naïve CD4+ T Cell Isolation Kit and EasySep™ Human Central and Effector Memory CD4+ T Cell Isolation Kit or **(B)** EasySep™ Human Naïve CD8+ T Cell Isolation Kit and EasySep™ Human Central and Effector Memory CD8+ T Cell Isolation Kit were stimulated with PMA/Ionomycin for six hours and treated with Brefeldin A four hours into stimulation. Cells were fixed, permeabilized, and stained for intracellular IL-2, IFNγ, and granzyme B. Production of IL-2, IFNγ, and granzyme B was assessed by flow cytometry. Production of granzyme B was not assessed in CD4+ T cell subsets. Data represents the mean (± standard deviation) of three independent experiments.

Summary

The new EasySep™ Human Central and Effector Memory CD4+ T Cell Isolation Kit and EasySep™ Human Central and Effector Memory CD8+ T Cell Isolation Kit:

- Facilitate the rapid isolation of both central and effector memory CD4+ or CD8+ T cell populations from the same sample
- Isolate cells that are functional and ready for downstream assays
- Are compatible for use with EasySep™ (Catalog #18000), "The Big Easy" EasySep™ (Catalog #18001), and EasyEights™ EasySep™ (Catalog #18103) magnets