

ISOLATION OF RARE ANTIGEN-SPECIFIC T CELL SUBSETS

Using Dextramer® Reagents and EasySep™ Release Technology

Overview

Isolating rare antigen-specific or unconventional T cell subsets presents a unique challenge in immunology research. In this protocol, we describe a high-efficiency method for labeling and isolating these cells using Dextramer® reagents from Immudex in combination with STEMCELL Technologies' [EasySep™ Release Positive Selection platform](#). This approach enables high-purity, particle-free enrichment of target T cells—offering a fast, reproducible solution that supports downstream applications such as flow cytometry, functional assays, single-cell analysis, TCR clonotyping, and expansion of enriched cells.

Background

The detection and isolation of antigen-specific or certain unconventional T cell subsets requires the use of specialized reagents, such as peptide-loaded major histocompatibility complex (MHC) multimers, to identify and label the target T cells. Dextramer® reagents from Immudex are MHC multimers that offer a significant improvement over other multimers, such as tetramers, as they provide an enhanced number of peptide-MHC complexes that boost the binding avidity and detection of even low-affinity T cells (Figure 1).

Dextramer® reagents can also be used to detect unconventional T cell subsets that cannot be identified by traditional cell-surface markers. One prime example is mucosal-associated invariant T (MAIT) cells expressing semi-invariant T cell receptors (TCRs) that require detection via MHC class I-related protein 1 (MR1) multimers loaded with microbial metabolite antigens. Another commonly targeted subset is invariant natural killer T (iNKT) cells that recognize the ligand α -Galactosylceramide (α -GalCer) presented on CD1d. Here, we describe an efficient and convenient method to isolate antigen-specific T cells labeled with Dextramer® reagents using [EasySep™ Release technology](#). EasySep™ Release combines the speed and ease of the EasySep™ cell isolation platform with the flexibility of the Releasable RapidSpheres™ for the separation of particle-free, highly purified immune cells.

Why Use EasySep™ Release?

EASY & EFFICIENT. Combine with Dextramer® labeling to isolate cells in as little as 45 minutes with minimal hands-on time.

PARTICLE-FREE & GENTLE. Obtain viable, functional cells without magnetic particles, columns, or cell sorting.

COMPATIBLE. Ideal for downstream applications such as flow cytometry, functional assays, single cell analysis, and TCR discovery.

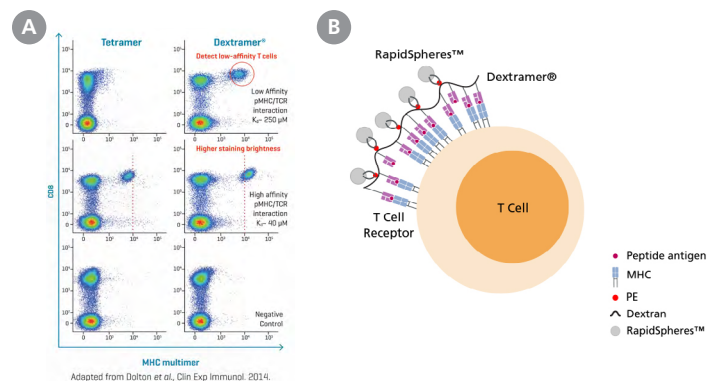


Figure 1. Dextramer® Reagents Combined with EasySep™ Technology Enable Enhanced Detection and Isolation of Antigen-Specific T Cells

(A) Flow cytometry analysis of antigen-specific CD8+ T cells using MHC tetramers (left) and Dextramer® reagents (right). Compared to tetramers, Dextramer® reagents enable detection of low-affinity T cells (pMHC/TCR Kd ~250 μM) and provide brighter staining of high-affinity interactions (pMHC/TCR Kd ~40 μM). This enhanced performance increases the sensitivity and specificity for identifying both low-affinity and rare T cell populations. A negative control is shown for comparison.

(B) Schematic of antigen-specific T cell detection using phycoerythrin (PE)-labeled Dextramer® (fluorophore-conjugated). Desired cells are first stained with Dextramer®, followed by labeling with EasySep™ Release PE Positive Selection Cocktail and magnetic particles before magnetic separation.

Materials

Immudex Dextramer® Products

Product	Description	Ordering
MR1 Dextramer® (PE-conjugated)	For isolating ligand-specific MAIT cells (e.g. 5-OP-RU)	MR1 Products
MHC I Dextramer® (PE-conjugated)	For isolating antigen-specific CD8+ T cells (e.g. CMV)	MHC I Products
CD1d Dextramer® (PE-conjugated)	For isolating iNKT cells reactive for α -GalCer	CD1d Products

For research use only. Not for use in diagnostic or therapeutic procedures.

STEMCELL Technologies EasySep™ Cell Separation Products

Product	Catalog #
EasySep™ Release Human PE Positive Selection Kit	17654
EasySep™ Buffer	20144
EasySep™ Magnet	18000
"The Big Easy" EasySep™ Magnet	18001

Protocol

Dextramer® reagents are provided conjugated to fluorochromes such as phycoerythrin (PE) to enable easy detection, and these fluorochromes can be targeted for immunomagnetic cell separation. The following protocol targets Dextramer®-labeled cells via PE using the [EasySep™ Release Human PE Positive Selection Kit](#) to isolate antigen-specific and particle-free T cells.

Note: Isolation performance is dependent on the target cell start frequency as very low target cell input may be difficult to isolate. (e.g. $\leq 0.05\%$ of total cells has been observed to lead to reduced performance).

1. Wash leukapheresis or peripheral blood mononuclear cell (PBMC) sample twice with recommended medium (EasySep™ Buffer or phosphate-buffered saline containing 2% fetal bovine serum with 1 mM EDTA). Centrifuge at $300 \times g$ for 5 mins,

determine cell concentration, and resuspend to 5×10^7 cells/mL. Ensure sample volume is 0.25 - 2 mL if using the purple [EasySep™ Magnet](#) or 0.5 - 8 mL if using the silver ["The Big Easy" EasySep™ Magnet](#). As a guideline, the data shown below used $2.5 - 5 \times 10^7$ total cells in the starting samples.

2. Transfer sample to a 5 mL or 14 mL round-bottom tube for the purple EasySep™ or silver "The Big Easy" magnets, respectively.
3. Add 10 μ L of Dextramer®-PE reagent to the sample and mix well. Incubate at room temperature (RT) in the dark for 10 mins.

Note: Higher sample volumes may require titration and optimization of the Dextramer® volume added or increased incubation times.

Optional: Stain additional samples with 10 μ L Dextramer®-PE reagent (or negative control Dextramer®) using the [MHC Dextramer® Staining Protocol](#) to assess the % of target cells in the starting sample (prior to enrichment). Keep cells on ice.

4. Add 50 μ L/mL EasySep™ Release PE Positive Selection Cocktail (Catalog #17654C). Mix well and incubate at RT for 5 mins.
5. Vortex EasySep™ Releasable RapidSpheres™ (Catalog #50201) and add 75 μ L/mL to the sample. Mix well and incubate at RT for 3 mins.
6. Top up the sample to 2.5 mL (purple EasySep™ Magnet) or 5.0 mL (silver "The Big Easy" EasySep™ Magnet; use 10.0 mL for samples > 3 mL) with recommended medium and place the tube in the magnet without the lid. Incubate at RT for 5 mins.
7. With the tube in the magnet, carefully pour off and discard the supernatant. If desired, this fraction may be retained for analysis to assess enrichment efficiency. Remove the tube from the magnet and resuspend the sample in the recommended medium. Repeat step 6 three more times (total of 4 x 5 min separations).
8. Prepare 1X EasySep™ Release Buffer by diluting 40X EasySep™ Release Buffer (Concentrate; Catalog #20165) using recommended medium.
9. After the final magnetic separation, resuspend the sample in 1X EasySep™ Release Buffer at the specified top-up volume recommended in the [EasySep™ Release Human PE Positive Selection Kit PIS](#). Incubate at RT for 1 min.
10. Place the sample tube in the magnet and incubate at RT for 5 mins. Pour off and collect the supernatant containing the released particle-free Dextramer®-PE+ target cells into a new tube. The cells are now ready for use.

Refer to the [Immudex MHC Dextramer® Staining Protocol](#) for downstream target cell quantification and surface marker characterization via flow cytometry. Note that for samples where 10 μ L of Dextramer® reagent was added during step 3 (above), no additional Dextramer® reagent was added during the subsequent staining procedure; only the start control samples were stained with additional Dextramer® reagent.

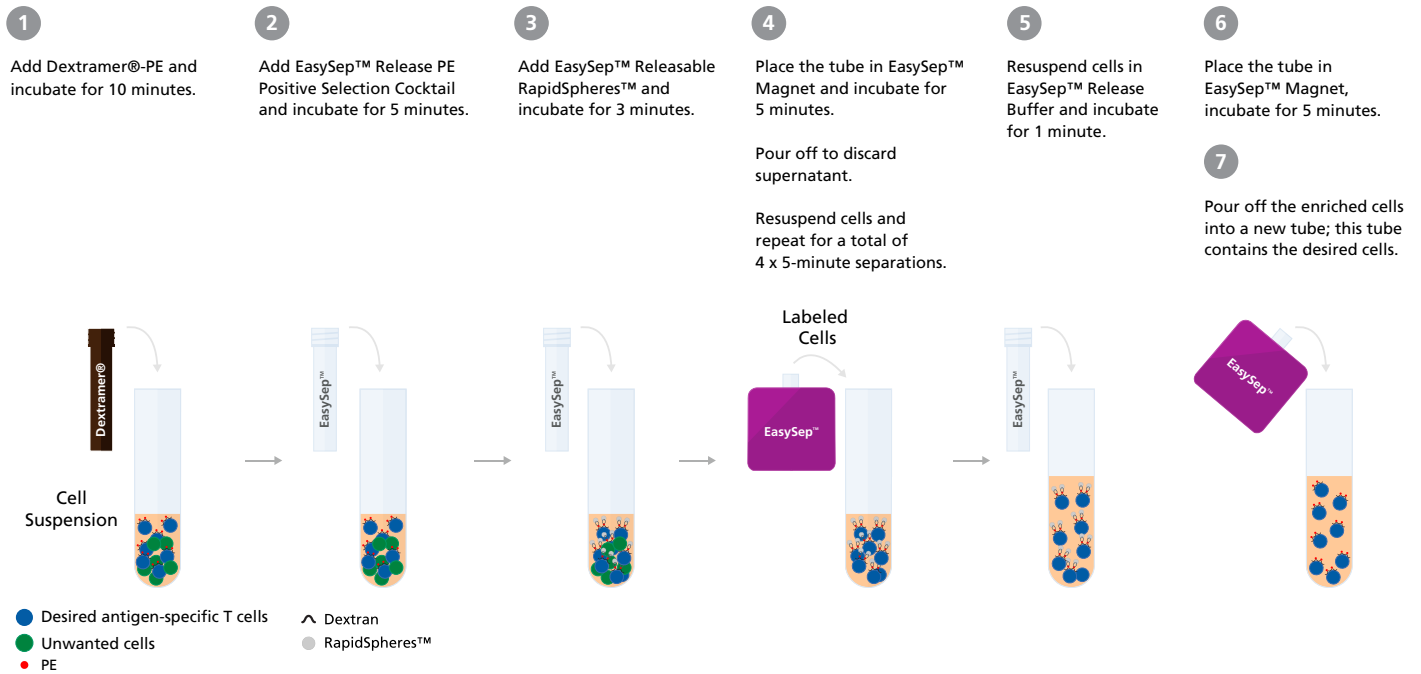


Figure 2. Workflow for Isolating Antigen-Specific T Cells Using Dextramer® Labeling and EasySep™ Release PE Positive Selection

Protocol for enriching antigen-specific T cells labeled with PE-conjugated Dextramer® reagents using the EasySep™ Release PE Positive Selection Kit. (1) Cells are labeled with PE-conjugated Dextramer® reagents for 10 minutes. (2) The EasySep™ Release PE Selection Cocktail is added to target PE-positive cells. (3) Releasable RapidSpheres™ are then added to magnetically label target cells. (4) The sample is placed in an EasySep™ magnet and the unlabeled fraction is poured off. This step is repeated for a total of 4 times to enhance purity. (5-7) The magnetic particles are removed using the Release Buffer, yielding particle-free, isolated, antigen-specific T cells ready for downstream applications.

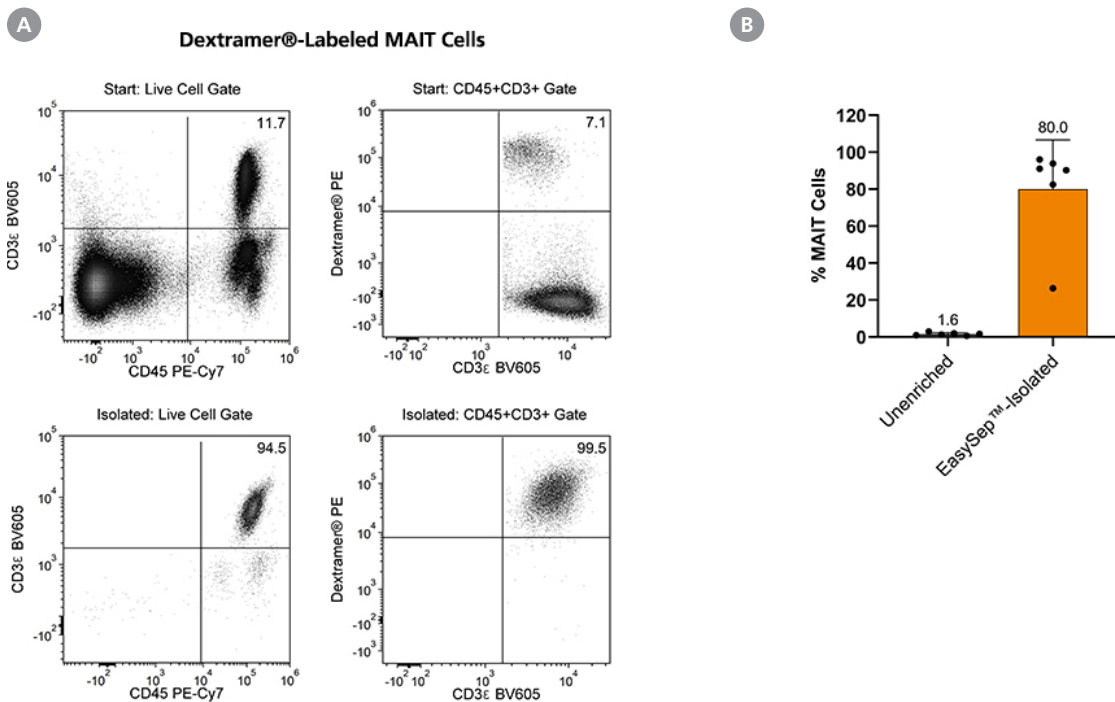


Figure 3. Isolation of Dextramer®-Labeled MAIT Cells Using EasySep™ Release Human PE Positive Selection Kit

(A) T cells (live CD45+CD3ε+) from leukopaks (5×10^7 cells in 1.0 mL) labeled with PE-conjugated MR1 Dextramer® (Catalog #ZA08004) before and after enrichment using EasySep™ Release Human PE Positive Selection Kit (Catalog #17654). Both total CD3ε+ cells and Dextramer®+ cells within the CD3ε+ population are shown and (B) Summary of 5-OP-RU-specific MAIT purities with an average total purity of 80.0%.

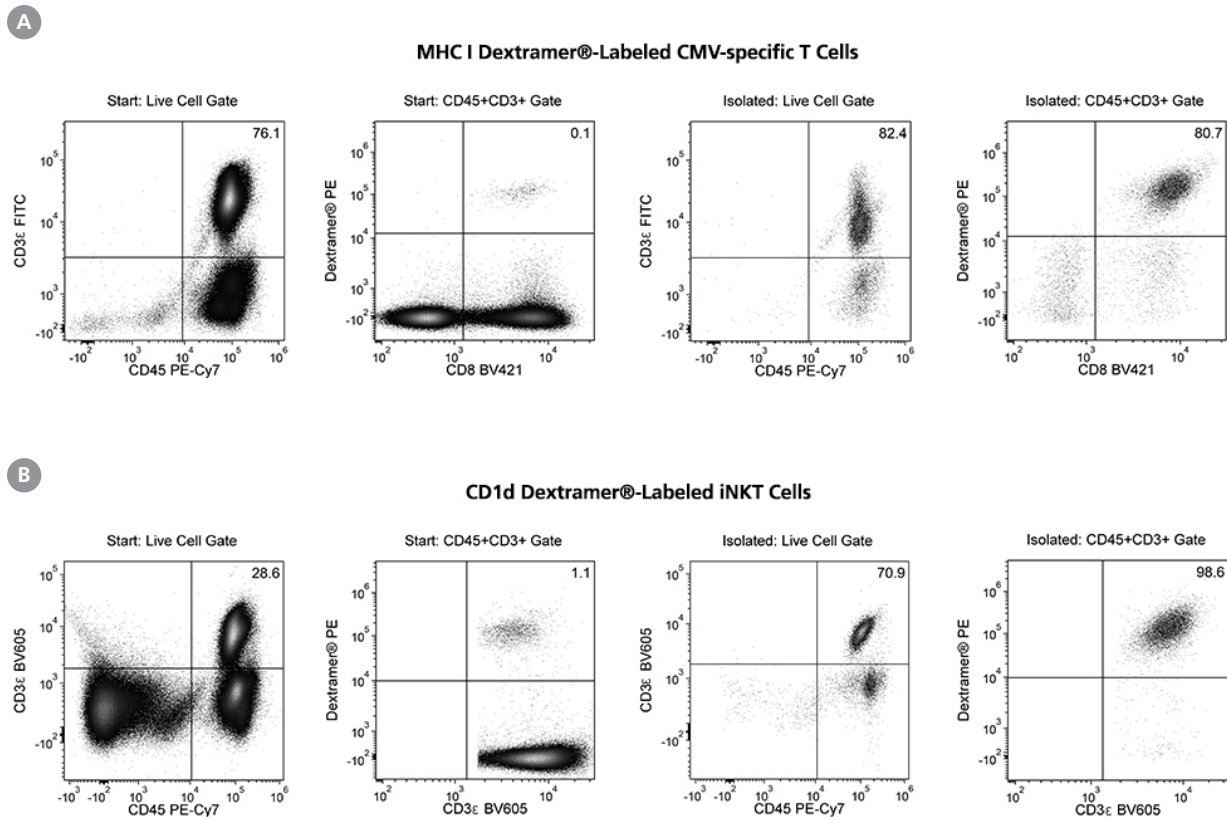


Figure 4. Isolation of CMV-Specific T cells and iNKT Cells Using EasySep™ Release Human PE Positive Selection Kit After Dextramer® Labeling

(A) T cells (live CD45+CD3ε+) from frozen PBMCs (4.15×10^7 cells in 0.83 mL) stained with PE-conjugated MHC I Dextramer® specific for CMV antigen (Catalog #WF02196) and (B) T cells from leukopaks (5×10^7 cells in 1.0 mL) stained with PE-conjugated CD1d Dextramer® loaded with α-GalCer (Catalog #XD08002) before and after enrichment using EasySep™ Release Human PE Positive Selection Kit (Catalog #17654). Both total CD3ε+ cells and Dextramer®+ cells within the CD3ε+ population are shown.

Conclusion

Detecting rare antigen-specific T cells can be challenging, and robust, sensitive detection requires specialized reagents such as Dextramers®. To purify these Dextramer®-labeled antigen-specific T cells, flow sorting can be replaced or supplemented with EasySep™ immunomagnetic cell separation. This approach efficiently and conveniently enriches the target cell populations while saving significant time compared to conventional methods.