TCR Dextramer® Staining Protocol

Products	Cat No. CSS008_4 TCR Dextramer®
Recommended use	Staining of peptide-pulsed T2 cells using fluorochrome-labelled TCR Dextramer® reagents.
Materials Provided	TCR Dextramer® FITC, PE, and/or APC
Materials Required (not provided)	T2 cells grown in exponential state at 0.1-1.0 million cells/mL in growth medium (see Procedural note A) Growth medium: IMDM + 10% FCS + 0.1% gentamycin Stain and wash buffer: PBS, 5% FCS, pH 7.4 TCR-matched peptide[B] dissolved to 200 µM in PBS, pH 7.4 or TCR-unrelated peptide dissolved to 200 µM in PBS, pH 7.4 4 mL Falcon disposable 12 x 75-mm test tubes or equivalent 96-well round-bottom microtiter plate Viability dye (e.g., FVS780 from BD, cat.no. 565388)
Procedure	A. Peptide-pulsing of T2 cells 1. Take T2 cells from an exponentially growing culture and wash them once in *stain and wash buffer*: a. Count T2 cells. Every condition (peptide sequence, concentration, etc.) requires 5.0 x 10^4 cells (1.5 x 10^5 cells for triplicates). Scale the number of cells below according to the specific assay setup b. Take a volume of the T2 cell suspension containing the required number of cells and centrifuge at 700 x g for 3 min. in a 4 mL tube. Discard supernatant c. Resuspend T2 cells in 2 mL *stain and wash buffer*. Centrifuge at 700 x g for 3 min. Discard supernatant d. Resuspend the cells in *stain and wash buffer* to a concentration of 5.0 x 10^5 cells/mL. 2. For each test condition, transfer 100 µL T2 cell suspension (5.0 x 10^4 cells) to a 4 mL tube. 3. Pulse T2 cells with peptide: a. Add 5 µL cognate peptide solution to each relevant test condition (final peptide concentration: 10 µM) b. Add 5 µL PBS (or control peptide solution) to each control condition c. Incubate at 37°C for 90 min. 4. Centrifuge T2 cells at 700 x g for 3 min and remove the supernatant carefully without disturbing the pellet

5. Resuspend cells in 2 mL **cold** (2-8°C) *stain and wash buffer.* Centrifuge at 300 x g for 5 min. and remove the supernatant. Proceed immediately to the next step.

B. Viability staining

6. Resuspend cells in buffer containing viability dye and incubate as specified by the supplier[C]
7. Wash cells in 2 mL **cold** (2-8°C) *stain and wash buffer.* Centrifuge at 300 x g for 5 min. and remove the supernatant. Proceed immediately to the next step.

C. Staining with TCR Dextramer®

8. Resuspend 2.5 x 10^6 cells/mL T2 cells in **cold** (2-8°C) *stain and wash buffer*
9. Distribute T2 cells in a 96-well round-bottom plate, 20 µL per well (5.0 x 10^4 cells)
10. Centrifuge the TCR Dextramer® at 10,000 x g for 1 min to avoid transferring any potential precipitate
11. Add 10 µL TCR Dextramer® (1 test) to each relevant well[D]
12. Incubate in the dark at **2-8°C** for 30 min.
13. Wash cells by adding 200 µL **cold** (2-8°C) *wash and stain buffer.* Centrifuge at 700 x g for 3 min. and remove the supernatant. Repeat washing for a total of 6 washes.
14. Resuspend the pellet(s) in the desired volume of **cold** *stain and wash buffer* suitable for your flow cytometer
15. Proceed to analyze the samples on a flow cytometer or store at 2-8°C in the dark. For optimal results, do not store the samples longer than 2 hours before acquisition.

Procedural notes

A. Staining with TCR Dextramer® can be performed on any cell suspensions or cell lines if the cells are non-fixed. Staining of cell lines other than T2 cells may require optimization of incubation time, temperature during peptide-pulsing and/or staining, and/or TCR Dextramer® reagent concentration
B. T2 cells express HLA-A*0201 at their surface and the TCR Dextramer® reagent should thus be directed against a peptide bound to HLA-A*0201
C. Viability staining may be performed at the beginning or end of staining procedure according to the manufacturer's instructions
D. Always keep TCR Dextramer® reagents stored at 2-8°C in the dark – the plastic vial only partially protects the reagents against light.

Technical support

For additional Tips & Tricks, FAQs and protocols, please visit https://www.immudex.com/resources/ or contact our support team at customer@immudex.com
Telephone: +45 3110 9292 (Denmark)

References

1. Sami, M. et al., Protein Eng Des Sel. 2007 Aug;20(8):397-403.

Analysis Guidelines

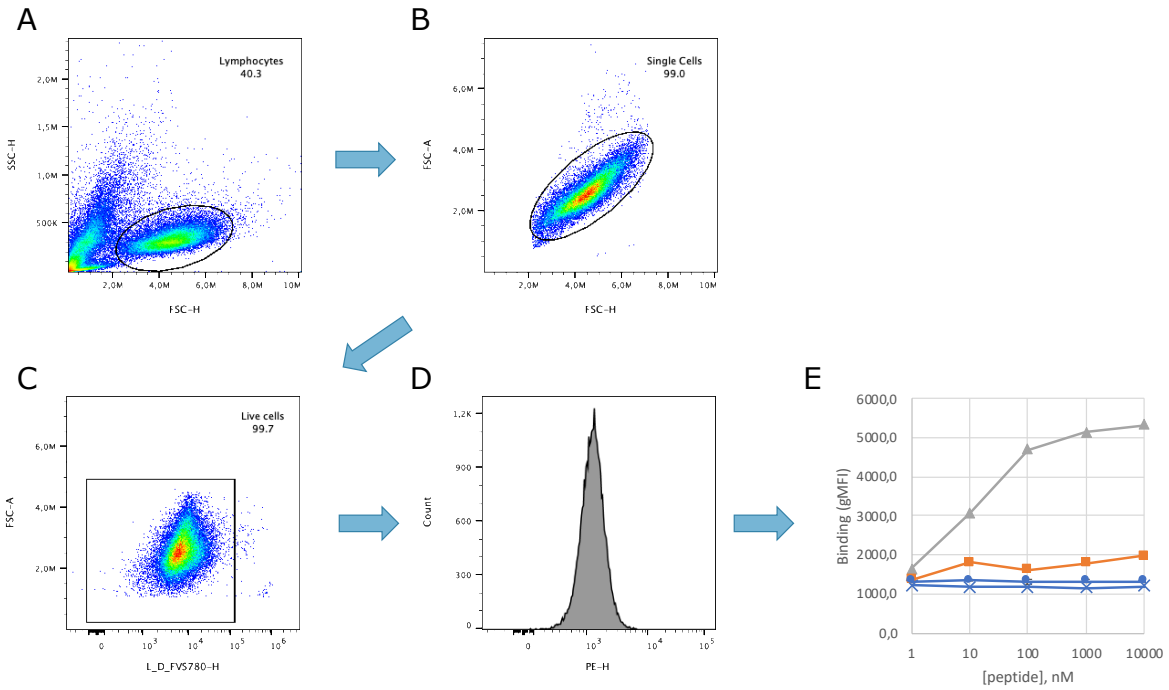

Figure 1: Example data for staining peptide-pulsed T2 cells with TCR Dextramer® reagents following the protocol outlined above. Gating strategy: (A) Lymphocytes were identified based on the forward (FSC) and side scatter (SSC) profile. (B) Next, single cells were selected by gating in a side scatter height (SSC-H) and side scatter area (SSC-A) profile plot. (C) Live cells were gated for further characterization based on the absence of staining with viability dye (FVS780). Analysis: (D) The mean fluorescence intensity of the T2 cells stained with TCR Dextramer® reagent was recorded at different peptide concentrations and plotted in (E). Two different TCR Dextramer® reagents were tested against their common, cognate SLLMWITQV peptide (grey/orange) as well as a negative control peptide (blue). TCR Dextramer® reagent 1 (grey) carries a very high-affinity TCR (Kd = 48 pM) while TCR Dextramer® reagent 2 (orange) carries a low affinity TCR (Kd = 32 µM)[1]. For both reagents, peptide-specific staining can be observed at a peptide concentration under 10 nM. Note: Staining intensity and sensitivity will generally depend on the TCR affinity for its cognate peptide as well as presentation levels on the peptide-pulsed cells.