

## Enumeration of CMV-specific T cells in whole blood

-using the CMV Dextramer package

### Principle

The CMV Dextramers of the CMV Dextramer package accurately detect and quantify CMV-specific T cells in blood samples. This involves a two-step procedure:

- Step 1: Determination of the percentage of CD3<sup>+</sup>CD8<sup>+</sup> CMV Dextramer<sup>+</sup> T cells in the sample
- Step 2: Determination of the absolute number of CD3<sup>+</sup>CD8<sup>+</sup> T cells in the sample

The actual number of CMV<sup>+</sup> CD3<sup>+</sup>CD8<sup>+</sup> T cells can then be determined.

### Materials

#### CMV Dextramer Package

HLA-A\*0101 (VTEHDTLLY)  
HLA-A\*0201 (NLVPMVATV)  
HLA-A\*0301 (KLGGALQAK)  
HLA-A\*2402 (QYDPVAAALF)  
HLA-B\*0702 (RPHERNGFTVL)  
HLA-B\*0702 (TPRVTGGGAM)  
HLA-B\*0801 (ELRRKMMYIM)  
HLA-B\*3501 (IPSINVHHY)  
Negative control

#### Ancillary reagents

Anti-CD8 antibody (e.g. FITC labeled)  
Anti-CD3 antibody (e.g. PcP labeled)  
Counting beads  
Lysing solution  
Fixing solution

### Staining protocol

#### **Tube A + B (Assessment of % CMV-specific T cells):**

1. Pipette 200 µl anti-coagulated whole blood in a 12x75 mm flow tube.
2. Add 10 µl appropriate CMV Dextramer (tube A) or negative control Dextramer (tube B) and incubate for 10 minutes at room temperature in the dark.
3. Add anti-CD8, anti-CD3 antibodies (e.g. labeled with FITC and PCP) in predetermined titers as recommended by the provider. Incubate for 30 min at 4°C in the dark.
4. Use appropriate lysis reagents to lyse red blood cells. Uti-Lyse from Dako and FACS Lyse from BD are known to work well together with the Dextramers.
5. Centrifuge 400 x g for 5 minutes, pour off supernatant and resuspend cell pellet in PBS

6. Centrifuge 400 x g for 5 minutes, pour off supernatant and resuspend cell pellet in 300-400 µl PBS with 2% Formaldehyde.
7. Store samples at 2-8°C in the dark until analysis on flow cytometer (samples can be run up to 24 hours after lysis).
8. Count 25.000 CD3 positive and CD8 positive events.

**Tube C (Assessment of absolute CD8 count):**

1. Add 100 µl anti-coagulated whole blood in a 12x75 mm flow tube.
2. Add anti-CD8, anti-CD3 and anti-CD4 antibodies in predetermined titers as recommended by the provider.
3. Incubate for 30 min at 4°C in the dark.
4. Use appropriate lysis reagents to lyse red blood cells.
5. Add suitable counting beads as recommended by the provider (both Accucount beads from Spherotech and BD's truecount tubes are known to work well).
6. Store samples at 2-8°C in the dark until analysis on flow cytometer (samples can be run up to 24 hours after lysis).
7. Count 10.000 Bead events.

**Data evaluation**

- 1) Determine percentage of CMV Dextramer-specific CD3<sup>+</sup>CD8<sup>+</sup> T cells in tube A. Use Tube B for defining negative threshold.
- 2) Calculate absolute counts of CD3<sup>+</sup>CD8<sup>+</sup> T cells in tube C. Use the equation:

$$\frac{\text{\# of CD3+CD8+ events counted in sample}}{\text{\# of beads counted in sample}} \times \frac{\text{\# of counting beads added}}{\text{Test volume (100 µl)}} = \text{Absolute count CD3+CD8+ cells}$$

- 3) Calculate absolute number of CMV-specific T cells in blood using the equation:

$$\text{\# CMV Dextramer-specific T cells} = \frac{(\text{Absolute count of CD3+CD8+ T cells (step 2)}) \times (\% \text{ CMV Dextramer-specific T cells (step 1)})}{100}$$