immudex
IMMUNOLOGY TAKEN TO A HIGHER LEVEL

Dextramer® CMV Kit	**Cat. No. GX01**
HLA-A*0101 / VTEHDTLLY / PE	Cat. No. WA02131G PE 25
HLA-A*0201 / NLVPMVATV / PE	Cat. No. WB02132G PE 50
HLA-A*0301 / KLGGALQAK / PE	Cat. No. WC02197G PE 25
HLA-A*2402 / QYDPVAALF / PE	Cat. No. WF02133G PE 25
HLA-B*0702 / RPHERNGFTVL / PE	Cat. No. WH02135G PE 25
HLA-B*0702 / TPRVTGGGAM / PE	Cat. No. WH02136G PE 25
HLA-B*0801 / ELRRKMMYM / PE	Cat. No. WI02137G PE 25
HLA-B*3501 / IPSINVHHY / PE	Cat. No. WK02138G PE 25
Negative control / PE	Cat. No. NI03233G PE 150

RUO (GMP): For research use only, manufactured under GMP. Not for use in diagnostic procedures

Recommended Use

Dextramer® CMV Kit is recommended for the identification and enumeration of cytomegalovirus (CMV)-specific CD8$^+$ T cells in cell samples by flow cytometry.

Background

Cytomegalovirus (CMV) is a herpes virus that infects 50-85% of the adult population and remains latent in healthy individuals through control by the presence of CMV-specific T cells. Reactivation of CMV is a frequently occurring complication of immunosuppression in transplant patients and other immune suppressed individuals and can significantly contribute to morbidity and mortality if the virus is not controlled.

CMV-specific CD8$^+$ T cells play a critical role in suppressing CMV reactivation. In healthy individuals an equilibrium is achieved where CMV-specific T cells control the persisting virus. When T cell function is impaired and equilibrium is not established, viral reactivation and clinical disease may develop.

Detection of CMV-specific CD8$^+$ T cells requires recognition of the T-cell receptor (TCR) by a unique combination of a MHC class I molecule coupled with a CMV-specific peptide. CMV-specific TCR's on the surface of CD8$^+$ T cells are recognized by complimentary CMV Dextramers. CMV Dextramers comprise dextran polymer backbone carrying multiple fluorochrome molecules (PE) and multiple MHC-peptide complexes displaying peptide epitopes from CMV antigens. CMV Dextramers can be used for detection and enumeration of CMV-specific CD8$^+$T cells by flow cytometry.

The Dextramer® CMV Kit comprises 8 different CMV Dextramers representing 7 different alleles covering ~95% of the European population.

Reagents provided

The Dextramer® CMV Kit comprises the following reagents:

HLA-A*0101 / VTEHDTLLY / PE	25 tests/0.25 ml	Cat. No. WA02131G PE 25
HLA-A*0201 / NLVPMVATV / PE	50 tests/0.50 ml	Cat. No. WB02132G PE 25
HLA-A*0301 / KLGGALQAK / PE	25 tests/0.25 ml	Cat. No. WC02197G PE 25
HLA-A*2402 / QYDPVAALF / PE	25 tests/0.25 ml	Cat. No. WF02133G PE 25
HLA-B*0702 / RPHERNGFTVL / PE	25 tests/0.25 ml	Cat. No. WH02135G PE 25
HLA-B*0702 / TPRVTGGGAM / PE	25 tests/0.25 ml	Cat. No. WH02136G PE 25
HLA-B*0801 / ELRRKMMYM / PE	25 tests/0.25 ml	Cat. No. WI02137G PE 25
HLA-B*3501 / IPSINVHHY / PE	25 tests/0.25 ml	Cat. No. WK02138G PE 25
Negative control / PE	150 tests/1.50 ml	Cat. No. NI03233G PE 150

The reagents are also available as individual reagents with 50 tests/0.50 ml per vial.

Storage and Preparation of Kit Components

Always keep CMV Dextramers stored at 2-8°C in the dark– the brown plastic vial does not protect the reagent sufficiently against light.

Precautions

For research use only. Not for use in diagnostic procedures

Specimens, before and after preparation, and all materials exposed to them, should be handled as if capable of transmitting infection and should be disposed of with proper precautions[7] .

CMV Dextramers contain sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

All materials should be disposed of according to your institution's guidelines for hospital waste disposal.

Suggested test procedure for enumeration of CMV-specific T cells in whole blood

Principle of procedure

The CMV Dextramers can be used to accurately detect and quantify CMV-specific T cells in whole blood samples. This involves a two-step procedure:

- Step 1: Determination of the percentage of CMV-specific $CD3^+CD8^+$ T cells in the sample (Tube A)
- Step 2: Determination of the absolute number of $CD3^+CD8^+$ T-cells in the sample (Tube C)

The absolute number of CMV-specific $CD3^+CD8^+$ T cells can then be determined (see Interpretation of Results below).

Reagents and buffers

Dextramer® CMV Kit (Cat. No. GX01)
Anti-CD8/FITC, e.g. clone SK1 (BD Cat. No. 345772)
Anti-CD4/PE, e.g. clone SK3 (BD Cat. No. 345769)
Anti-CD3/PerCP, e.g. clone SK7 (BD Cat. No. 345766)
Truecount tubes (BD Cat. No. 340334)
FACS Lysing Solution (10X) (BD, Cat. No. 349202).
PBS (e.g. 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.47 mM KH_2PO_4; pH = 7.4)
Fixing solution (e.g. 2% Methanol free formalin in PBS)

Assay Procedure
Select a CMV Dextramer matching the HLA-type of the patient. If multiple CMV Dextramers are applicable, select all and make analysis for each allele.

Collect blood by venipuncture into a blood collection tube containing an appropriate anti-coagulant (EDTA, Citrate or Heparin). Collected blood should be analyzed within 24 hours.

Prepare FACS Lysing Solution: Dilute the FACS Lysing Solution (10X concentrate) 1:10 with room temperature (20° to 25°C), deionized water. Store prepared solution as recommended by the manufacture.

Stain cells as described for tube A, B and C as described below:

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

1. Pipette 200 µl anti-coagulated whole blood in a 12 x 75 mm flow tube.

2. Add 10 µl appropriate CMV Dextramer to Tube A and 10 µl Negative control / PE to Tube B and incubate for 10 min. at room temperature in the dark. If a blood sample is analyzed by more than one CMV Dextramer, prepare separate Tube A for each CMV Dextramer.

3. Add 10 µl anti-CD8/FITC and 10 µl anti-CD3/PerCP to both Tube A and Tube B.

4. Incubate for 30 min. on ice in the dark.

5. Add 2 mL of 1x FACS Lysing Solution. Vortex gently and incubate for 10 min. in the dark at room temperature.

6. Centrifuge 400 x g for 5 min., pour off supernatant and resuspend cell pellet in 2 ml PBS.

7. Centrifuge 400 x g for 5 min., pour off supernatant and resuspend cell pellet in 300-400 µl Fixing solution.

8. Store samples at 2-8°C in the dark until analysis on flow cytometer (samples can be run up to 24 hours after lysis).

9. Acquire 25.000 dual $CD3^+$ and $CD8^+$ events.

Tube C (Assessment of absolute count of $CD3^+CD8^+$ cells):

1. Add 100 µl anti-coagulated whole blood in a TruCOUNT Tube.

2. Add 10 µl anti-CD8/FITC, 10 µl anti-CD4/PE and 10 µl anti-CD3/PerCP.

3. Incubate for 30 min. at 2-8°C in the dark.

4. Add 1 mL of 1x FACS Lysing Solution. Vortex gently and incubate for 10 min. in the dark at room temperature.

5. Store samples at 2-8°C in the dark until analysis on flow cytometer (samples can be analyzed up to 6 hours after lysis).

6. Acquire 10.000 bead events, using a threshold set on $CD3^+$ cells.

Acquisition protocols
Before acquiring samples adjust the threshold to include cell and bead populations of interest and minimize debris. Use same instrument settings for Tube A, B and C.

Make protocols that allow the following dot plot figures to be made:

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

A) FS vs. SS: Ensure lymphocyte population is visible. Draw exclusion gate on low scatter debris (region R1)
B) Anti-CD3 vs. SS: exclude region R1, draw gate around $CD3^+$ cells (region R2)
C) FS vs. SS: exclude region R1, include region R2, draw gate on lymphocytes (region R3)
D) Anti-CD3 vs. anti-CD8: exclude region R1, include region R2 + R3, draw gate on $CD8^+$ cells (region R4)
E) CMV Dextramer vs. anti-CD8: exclude region R1, include region R2 + R3 + R4, draw gate around CMV^+ cells (region R5)

Acquire 25.000 $CD3^+CD8^+$ events in region R4.

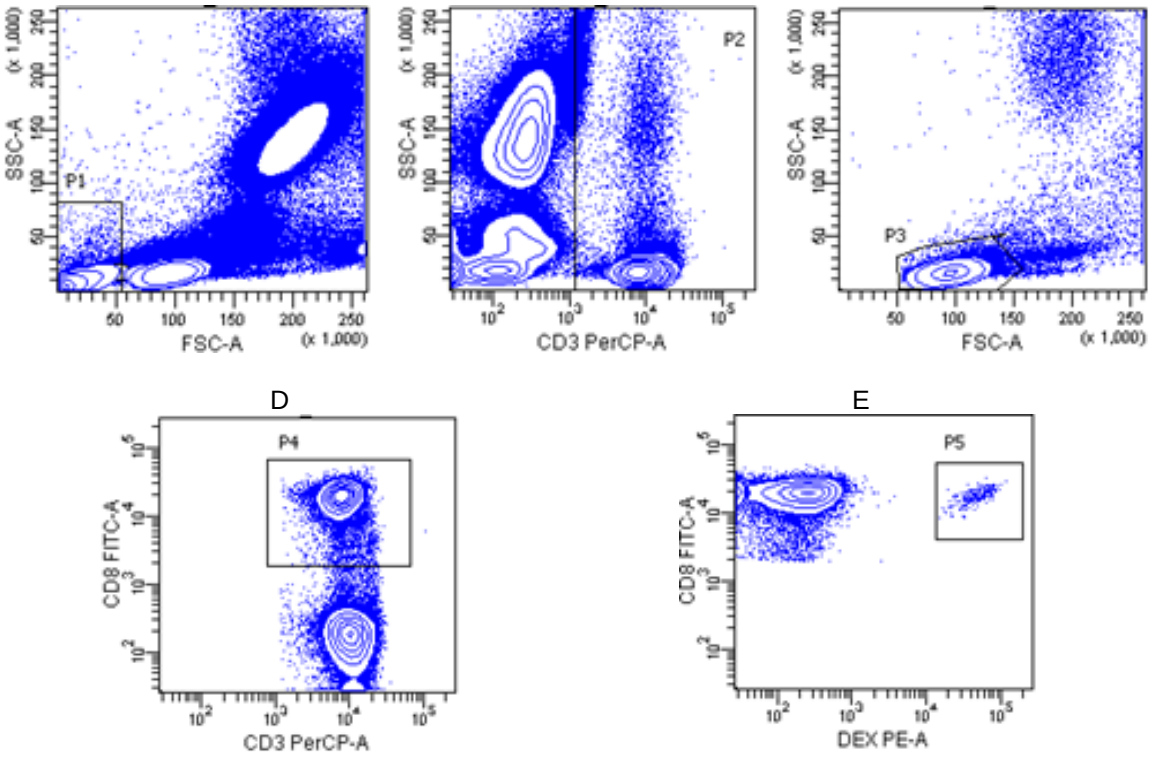

Figure 1. Dot plots tube A and B. Illustrative example from FACSCanto II using Diva software

Tube C (Assessment of absolute count of $CD3^+CD8^+$ cells):

F) Anti-CD3 vs. SS: Set a threshold excluding CD3 negative events. Ensure whole population of both beads and lymphocytes are visible in included area. Draw gate on $CD3^+$ cells (region R6)
G) FS vs. SS: include region R6, draw gate on lymphocytes (region R7)
H) Anti-CD3 vs. anti-CD8: include region R6 + R7, draw gate on $CD8^+$ cells (region R8)
I) Anti-CD4 vs. anti-CD8: Ungated, draw gate on bead events (region R9).

Acquire 10.000 bead events in region R9.

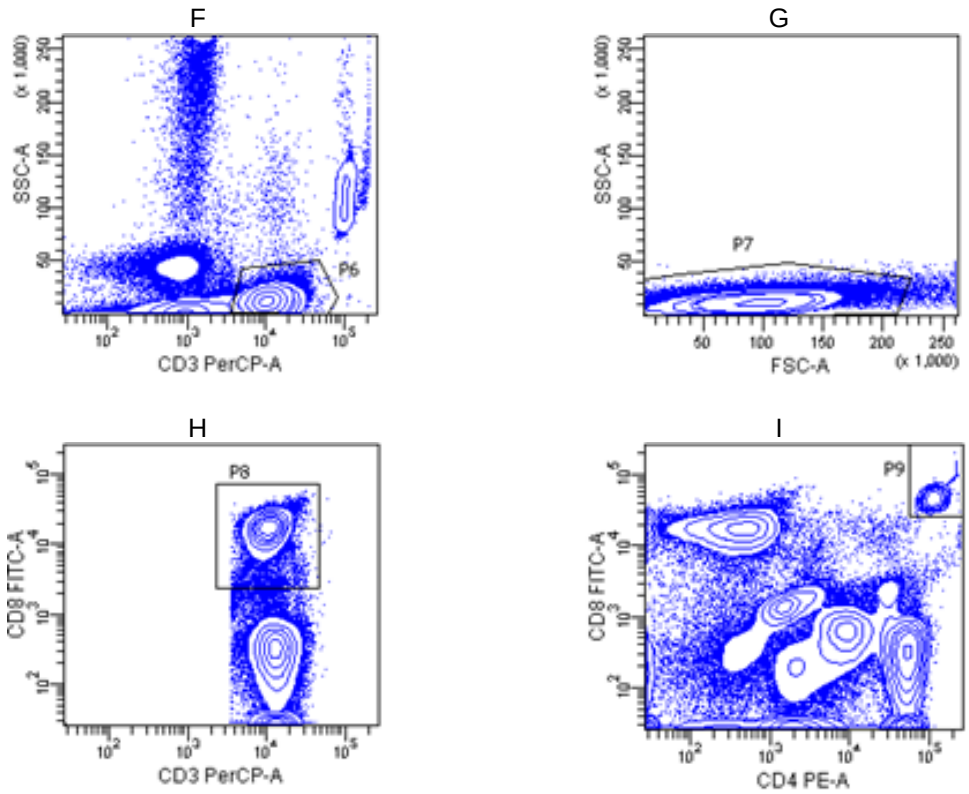

Figure 2. Dot plots tube C. Illustrative example from FACSCanto II using Diva software

Interpretation of Results
Determine the following values:

Value	Tube	Region	Purpose
# $CD3^+CD8^+$	C	R8, plot H	Calculation of absolute count of $CD3^+CD8^+$ cells/ µl blood
# Bead events	C	R9, plot I	Calculation of absolute count of $CD3^+CD8^+$ cells/ µl blood
%$CD3^+CD8^+$	C	R8, plot H	Reproducibility check between Tube C and Tube A
%$CD3^+CD8^+$	A	R4, plot D	Reproducibility check between Tube C and Tube A
%$CD3^+CD8^+CMV^+$	A	R5, plot E	Calculation of absolute count of $CD3^+CD8^+CMV^+$ cells/ µl
%$CD3^+CD8^+CMV^+$	B	R5, plot E	Determination of background staining

1) Calculate absolute counts of $CD3^+CD8^+$ T cells in Tube C. Use the equation:

$$\text{Absolute count } CD3^+CD8^+ \text{ cells} = \frac{CD3^+CD8^+ \text{events (region R8)}}{\text{bead events (region R9)}} \text{x} \frac{\text{bead events per test}}{\text{whole blood volumen tested (µl)}}$$

2) Determine percentage of CMV-specific $CD3^+CD8^+$ T cells in Tube A:

% CMV Dextramer positive events in region R5

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

$$\text{Absolute count } CMV^+ \text{ T cells} = \frac{(\text{absolute count } CD3^+CD8^+ \text{cells (step 1)}) \text{x (\% CMV Dex}^+\text{T cells (step 2)})}{100}$$

Recommended Quality Control

Flow cytometer: Follow manufacturer recommendations for daily flow cytometer instrument set-up and daily instrument quality assurance for three-color immunophenotyping[5].

Method: Use commercial whole blood controls providing established values for percent positive $CD4^+$ and $CD8^+$ cells with each run to assess system performance. The control cells should be stained as described for Tube C. The values of the two subsets must fall within the expected range stated by the provider.

Control between tubes: $CD8^+$ results expressed as a percentage of $CD3^+$ should be $\leq$ 5% between Tube A and Tube C.

Background: Tube B is used to evaluate background. The percentage of Dextramer-specific T cells should be <0.2% of $CD8^+$ T cells.

Procedural Notes
The addition of a precise volume of blood is critical to achieve reliable results. Use electronical pipettes that operate in the reverse pipetting mode or perform the reverse pipetting technique using manual pipettes.

Troubleshooting

If unexpected results are observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our customer services

Technical Advice and Customer Service

For all inquiries, please contact Immudex

Manufactured by:

Immudex ApS
Bredevej 2A
DK-2830 Virum
Denmark

Phone: +45 3110 9292
E-mail: customer@immudex.com