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Dextramer® CMV Kit **Cat. No. CX03**

Intended Use

Dextramer® CMV Kit is a semi-quantitative assay intended for the identification and enumeration of cytomegalovirus (CMV)-specific CD8+ T cells in anticoagulated whole blood specimens by flow cytometry.

Dextramer® CMV Kit is indicated for assessment of CMV-specific immune status and risk of CMV reactivation in adult human stem cell transplant patients following immunosuppression and used in conjunction with other laboratory and clinical findings.

The kit cannot be used to measure CMV infection or disease.

The kit is limited to individuals with the following HLA types: A*0101, A*0201, B*0702, B*0801, B*3501.

Summary and Explanation

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. 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.

Reactivation of CMV is a frequently occurring complication of immunosuppression in transplant patients and can significantly contribute to morbidity and mortality if the virus is not controlled.

CMV reactivation may be controlled by preemptive antiviral therapy, but this is often hampered by side-effects, renal toxicity and drug-resistant strains.

Several studies have demonstrated an increased risk of CMV viremia or disease in patients with low levels of specific T cell immunity, and likewise, the development of T cell immunity has been shown to be associated with decreased risk of CMV viremia and disease after transplantation[1-4, 8]

Enumeration of CMV-specific $CD8^+$ T cells provide information about the status of the CMV-specific immune response and can predict patients in high risk of developing CMV disease[1-3]

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 CMV Dextramers. CMV Dextramers comprise dextran polymer backbone carrying multiple MHC-peptide complexes and fluorochrome molecules (PE). Together with fluorescent-labeled anti-CD3 and anti-CD8 antibodies CMV Dextramers are used for detection and enumeration of CMV-specific $CD3^+CD8^+$T cells by flow cytometry[9].

T cell immune response to CMV varies between individuals, is dependent on HLA-type, and is influenced by HLA composition. CMV-specific cellular immune responses restricted by some alleles dominate those restricted by others[6]. It is therefore important to measure CMV-specific immune responses restricted by as many HLA alleles as possible in a given individual.

The Dextramer® CMV Kit comprises 5 different CMV Dextramers representing 5 different alleles.

Principle of Procedure

The CMV Dextramers accurately detect and quantify CMV-specific T cells in 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/µl can then be calculated (see Calculation of Results below).

Materials

Materials provided
The Dextramer® CMV Kit comprises the following reagents:

CMV Dextramer reagents		
HLA-A*0101 / VTEHDTLLY / PE	25 tests/0.25 ml	Cat. No. CA2131-PE
HLA-A*0201 / NLVPMVATV / PE	50 tests/0.50 ml	Cat. No. CB2132-PE
HLA-B*0702 / TPRVTGGGAM / PE	25 tests/0.25 ml	Cat. No. CH2136-PE
HLA-B*0801 / ELRRKMMYM / PE	25 tests/0.25 ml	Cat. No. CI2137-PE
HLA-B*3501 / IPSINVHHY / PE	25 tests/0.25 ml	Cat. No. CK2138-PE
Negative control / PE	100 tests/1.00 ml	Cat. No. CI3233-PE

Each CMV Dextramer is a PE-labeled dextran coupled with MHC molecules complexed with CMV peptide in buffered saline containing 1% BSA, 15 mM NaN_3, pH 7.2.

Antibodies		
Anti-CD8/FITC	3 ml	Cat. No. A-CD8-FITC
Anti-CD3/PerCP	3 ml	Cat. No. A-CD3-PerCP
Anti-CD4/PE	2 ml	Cat. No. A-CD4-PE

Materials required but not provided
Flow tube, 12 x 75 mm, disposable tubes recommended for flow cytometry
Trucount 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)
Commercial CD3/CD4/CD8 cell control with established values for percent positive and absolute counts

Instruments required but not provided
Flow cytometer, for in vitro diagnostics use, with excitation light source and 3 independent fluorescence channels for FITC, PE and PerCP, and capable of forward scatter (FS) and side scatter (SS) detection.
Centrifuge capable of 400 x g
Pipette

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.

Kit components are provided with expiration date on the label. Do not use kit components beyond their expiry.

Precautions

For *in vitro* diagnostic use.

For professional users.

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.

Specimen Collection and Preparation

Collect blood by venipuncture into a blood collection tube containing an appropriate anti-coagulant (EDTA or Heparin). Collected blood should be stored upright at room temperature and analyzed within 48 hours.

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.

Test Procedure

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

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, mix 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, mix.
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 and mix.

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 24 hours after lysis).

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

Quality Control

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

Method: Use commercial whole blood controls providing established values for percent $CD4^+$ 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.

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 P1)
B) Anti-CD3 vs. SS: exclude region P1, draw gate around $CD3^+$ cells (region P2)
C) FS vs. SS: exclude region P1, include region P2, draw gate on lymphocytes (region P3)
D) Anti-CD3 vs. anti-CD8: exclude region P1, include region P2 + P3, draw gate on $CD8^+$ cells (region P4)
E) CMV Dextramer vs. anti-CD8: exclude region P1, include region P2 + P3 + P4, draw gate around CMV^+ cells (region P5)

Acquire 25.000 $CD3^+CD8^+$ events in region P4.

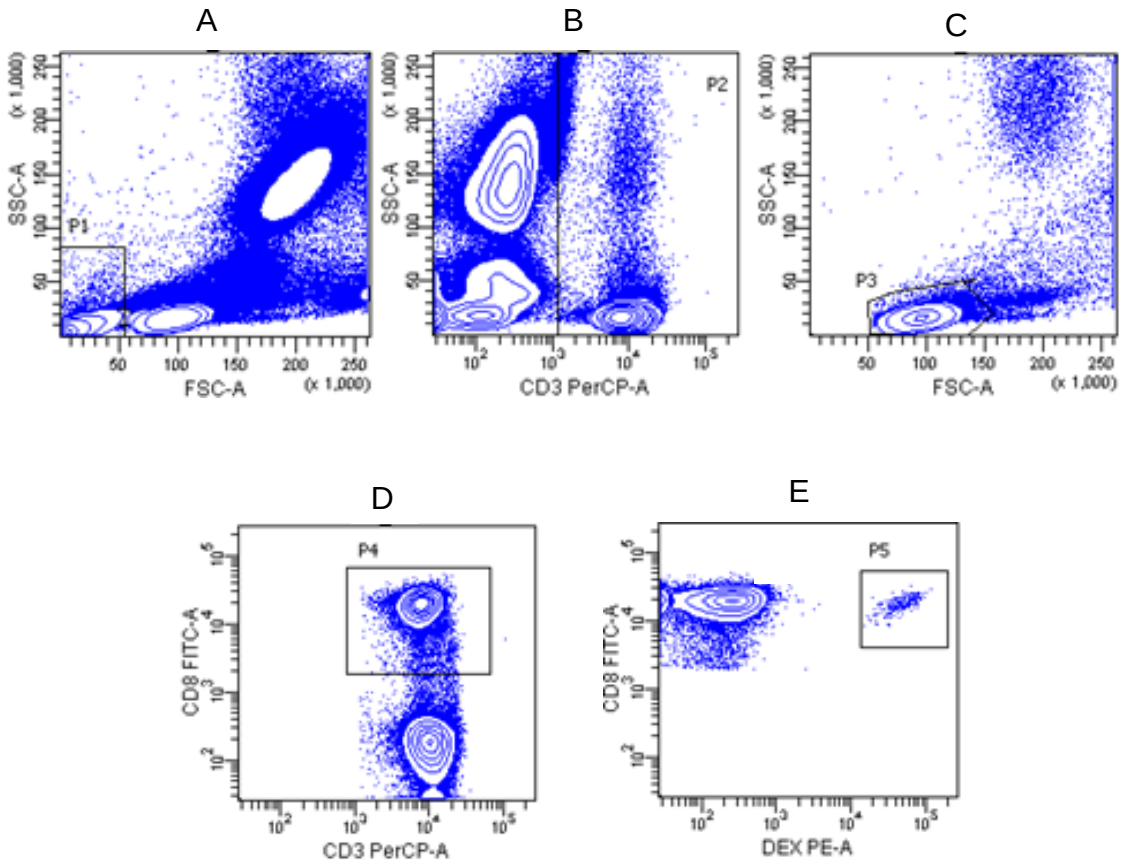

Figure 1. Dot plots tube A and B. Illustrative example from FACSCanto II using Diva software

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 P6)
G) FS vs. SS: include region P6, draw gate on lymphocytes (region P7)
H) Anti-CD3 vs. anti-CD8: include region P6 + P7, draw gate on $CD8^+$ cells (region P8)
I) Anti-CD4 vs. anti-CD8: Ungated, draw gate on bead events (region P9).

Acquire 10.000 bead events in region P9.

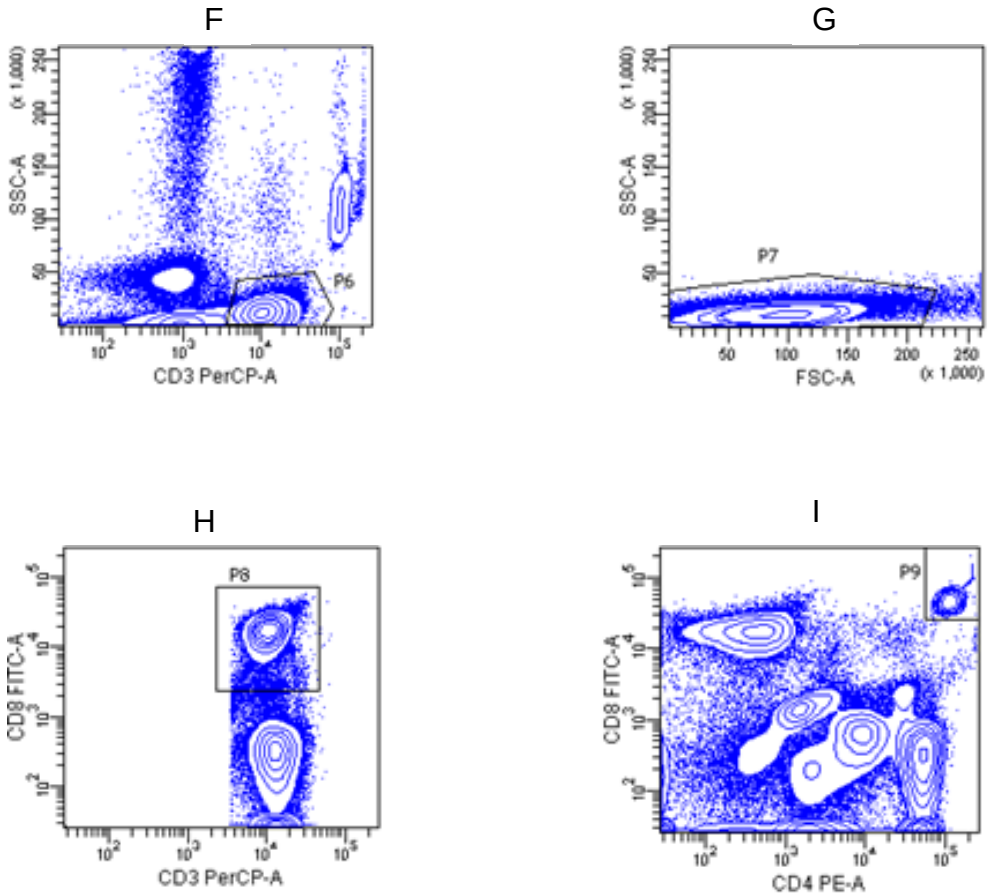

Figure 2. Dot plots tube C. Illustrative example from FACSCanto II using Diva software

Calculation of Results

Determine the following values:

Value	Tube	Region	Purpose
# $CD3^+CD8^+$	C	P8, plot H	Calculation of absolute count of $CD3^+CD8^+$ cells/ µl blood
# Bead events	C	P9, plot I	Calculation of absolute count of $CD3^+CD8^+$ cells/ µl blood
%$CD3^+CD8^+$	C	P8, plot H	Reproducibility check between Tube C and Tube A
%$CD3^+CD8^+$	A	P4, plot D	Reproducibility check between Tube C and Tube A
%$CD3^+CD8^+CMV^+$	A	P5, plot E	Calculation of absolute count of $CD3^+CD8^+CMV^+$ cells/ µl
%$CD3^+CD8^+CMV^+$	B	P5, plot E	Determination of background staining

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

   % CMV Dextramer positive events in region P5

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

$$\text{Absolute count } CD3^+CD8^+ \text{ cells} = \frac{CD3^+CD8^+ \text{events (region P8)}}{\text{bead events (region P9)}} \text{ x } \frac{\text{bead events per test}}{\text{whole blood volumen tested (µl)}}$$

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

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

Interpretation of Results

Results are reported as the number of CMV-specific $CD8^+$ T cells/ µl blood (absolute count).

CMV-specific $CD8^+$ T cell counts should be measured pre-transplant and sequentially post-transplant starting Day 30 and interpreted in conjunction with other clinical and laboratory information, such as CMV viral load or CMV antigenemia measurements in order to evaluate CMV immune status and risk of CMV reactivation.
Patients with prior CMV viremia that develop CMV-specific CD8+ T cell count ≥7 cells/µl by Day 100 as measured by Dextramer® CMV Kit are at lower risk for recurrence of CMV viremia between Days 100 -365 post-transplant relative to patients with <7 cells/µl. (see Clinical studies below.)

Limitations

The use of the Dextramer® CMV Kit is limited to individuals with at least one of the following HLA types: A*0101, A*0201, B*0702, B*0801, B*3501. It cannot be used for individuals with HLA-type different from those.

Patients being treated with antithymocyte globulin (ATG) may experience delayed immune recovery (8).

The Dextramer® CMV Kit does not measure CMV infection or disease.

Individual CMV Dextramers can only be used on samples with matching HLA-types.

Predictive absolute counts may vary in different patient populations and can be affected by treatment types and schedules. Therefore, it is recommended that laboratories evaluate their optimal predictive cut off.

Performance Characteristics

Analytical performance

Analytical sensitivity
The functional assay sensitivity is 1 cells/µl as determined by the lowest concentration of cells (cells/µl) that can be determined with a CV% below 20%.

Analytical specificity
100% (35/35) routine blood specimens from CMV seronegative stem cell transplant recipients showed 0.00 – 0.06 cells/µl and thus below the functional assay sensitivity of 1 cell/µl

Inter-lab reproducibility
The inter-lab reproducibility CV ranged between 6% and 18%.

Intra-lab reproducibility

The intra-lab reproducibility CV ranged between 5% and 14%.

Linearity
The Dextramer CMV assay showed linearity in the range of 1-107 CMV-specific T cells/µl with a slope ranging between 0.98-1.08.

Interference
There was no significant interference from the tested cell populations equivalent to 2x normal level for monocytes, equivalent to 3x normal level for granulocytes, equivalent to 3x normal level for platelets, and equivalent to 2x normal level for red blood cells.

Cross-reaction
No significant cross-reaction with allele mismatching CMV Dextramer reagents was observed. All results from analysis of 4 blood samples with CMV-specific T cells with HLA mis-matched CMV Dextramer were within 0.00 – 0.11 cells/µl and thus below the functional assay sensitivity of 1 cell/µl.

Clinical performance

One hundred twenty allogeneic patients post-SCT were followed for up to one year for recurrence of CMV infection and determination of numbers of CMV-specific CD8+ T cells using the Dextramer CMV Kit. Dextramer analysis were performed pre-transplant at day 30, 100 and 360 and included the alleles HLA-A*0101, A*0201, B*0702, B*0801 and B*3501. The Dextramer CMV Kit measurement giving highest absolute counts at each timepoint for each patient was used in analysis and evaluation.

Of the 120 original patients, 68 patients were CMV seropositive (recipient and/or donor) prior to transplantation. 34 of these 68 patients had evidence of CMV reactivation through day 100 post transplant; 5 of these patients did not have Dextramer® CMV Kit measurements at Day 100 and were excluded from analysis, leaving 29 patients in the analysis population.

Results for Dextramer® CMV Kit testing of the 29 subjects with CMV reactivation between Days 0 -100 are shown in the table below. In summary, the relative risk in developing antigenemia is 3.4 (95% CI: 1.57 – 7.46) for patients with < 7 cells/µL CMV-specific CD8+ T cells determined by the Dextramer CMV kit, as compared to patients with ≥ 7 cells/µL CMV-specific CD8+ T cells.

Day 100	Development of antigenemia (post Day 100)		
# CMV+ T cells	Yes	No	Total
<7 cells/ul	9	1	10
≥7 cells/ul	5	14	19
Total	14	15	29
Risk	3.4 (95% CI: 1.57 – 7.46)		

Summary of Safety and Performance

The summary of safety and performance (SSP) will be made available on https://ec.europa.eu/tools/eudamed where it is linked to the Basic UDI-DI:

5714183DEXCMVU5. A copy of the SSP can also be requested by contacting Immudex at customer@immudex.com.

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 service

Serious Incidents

If any serious incident occurs in relation to the device, immediately contact Immudex and the competent authority of your country.

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Technical Advice and Customer Service

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Revision History

Revision	Date of Issue	Modification
TF1010.07	20170607	Latest IVDD version (CX01)
TF1293.01	20230727	Update to IVDR, incl. alignment with US FDA cleared device (CX02)