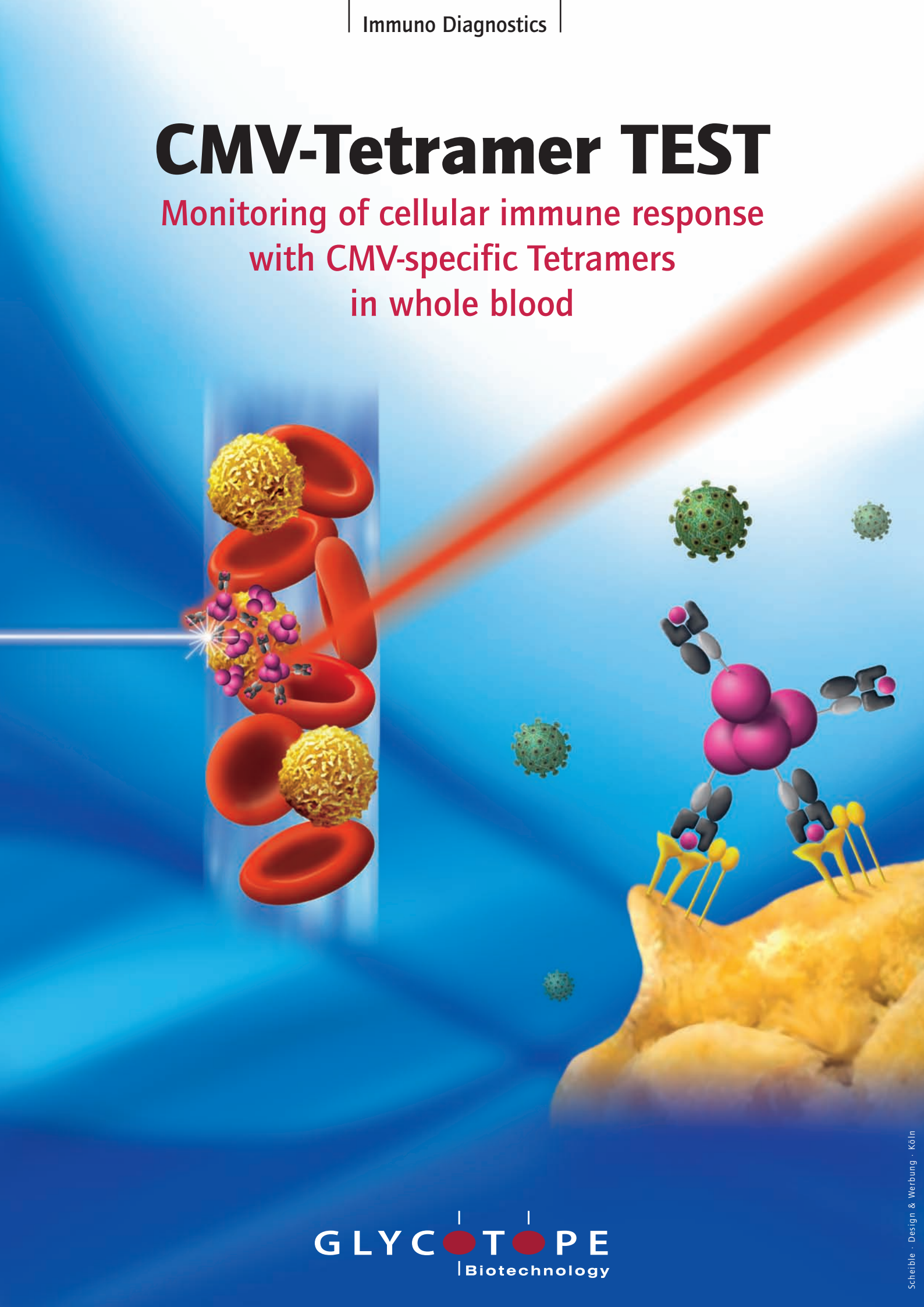
CMV-Tetramer TEST

Monitoring of cellular immune response with CMV-specific Tetramers in whole blood



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Monitoring CMV-specific CD8+ T cell numbers – a 2 step procedure

Step 1: Assessment of absolute CD8+ T cell counts using counting beads

1. Addition of antibodies

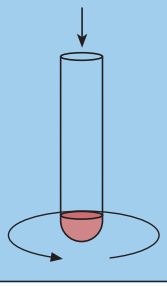
add CD3-PerCp, CD8-FITC, CD4-PE
5 µl each

Counting Tube

The lyophilisate of the counting beads is dissolved by addition of the antibodies

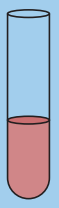
2. Addition of whole blood

+ 100 µl EDTA whole blood, vortex and incubate 20 min at RT

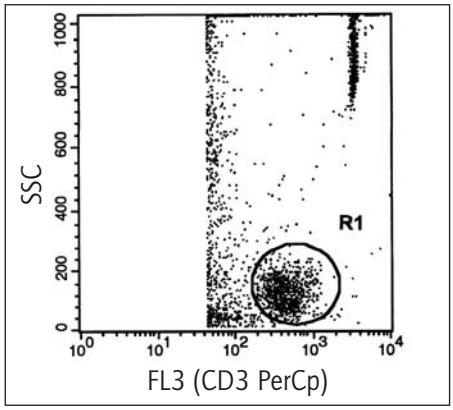


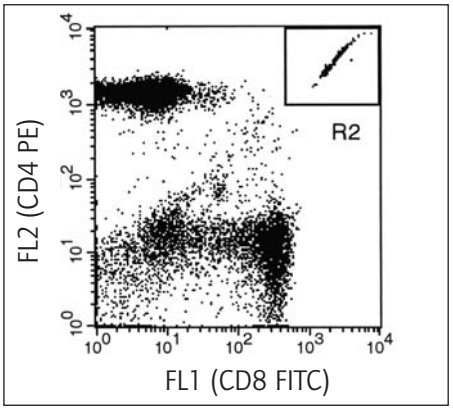
3. Lyse – no wash

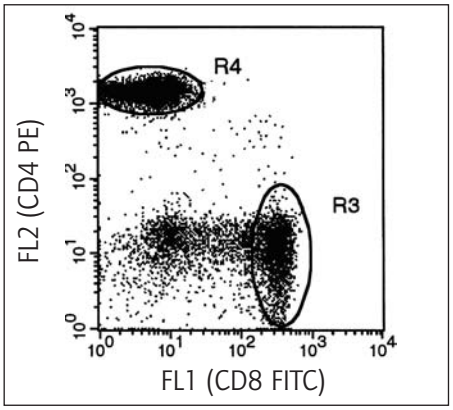
+ 2 ml LYSING SOLUTION



Vortex and incubate for 15 min in the dark at RT
Do not wash
Perform Analysis







$$\frac{\text{\# of CD3/CD8 events in R3}}{\text{\# of beads in R2}} \times \frac{\text{\# of beads per test*}}{\text{test volume (100 µl)}} = \text{absolute count of CD3/CD8 cells/µl}$$

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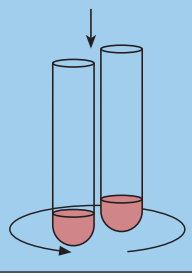
Step 2: Assessment of % Tetramer binding T-lymphocytes

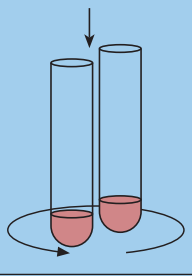
1. Addition of whole blood 200 µl
2. Addition of Tetramer

add 10 µl CMV-tetramer to tube 1 and 10 µl negative Tetramer to tube 2 – vortex and incubate 30 min at RT



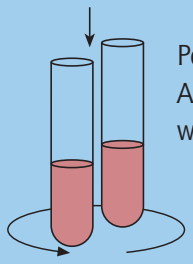
3. Addition of CD3 and CD8

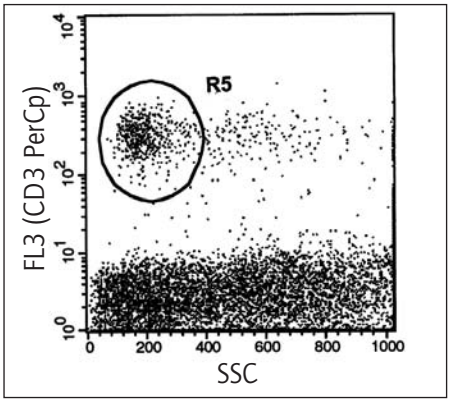
add 5 µl CD3-PerCp + 5 µl CD8-FITC – vortex – stain 20 min at RT in the dark

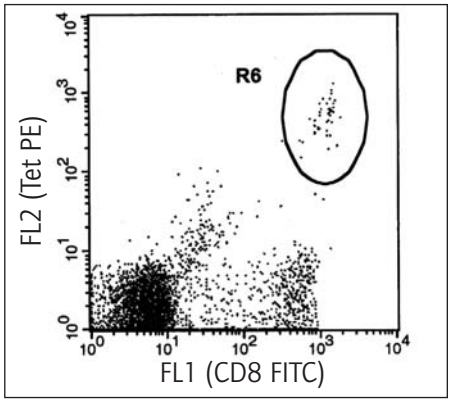


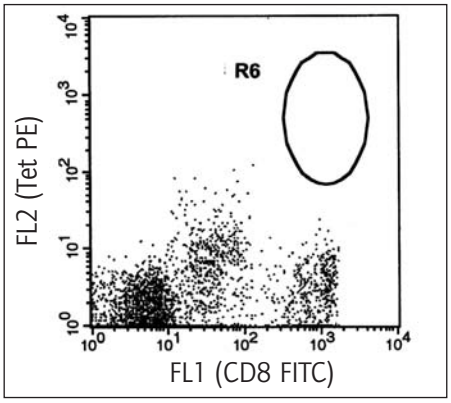
4. Lyse – <u>wash</u>

+ 2 ml LYSING SOLUTION, vortex and incubate 20 min at RT, spin at 250 x g and wash twice with 1 ml PBS









Absolute Count of CD3+CD8+ cells x Tetramer-positive events as a percent of CD8+ cells/100

= CD3+CD8+ Tetramer+ cells per µl whole blood

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