In situ detection of antigen-specific T cells in frozen tissue sections

Guideline for staining acetone fixated cryo-sections

The optimal concentration of Dextramer reagent may vary more than ten-fold among applications, and staining of sections from cryo-preserved tissue with MHC Dextramers may require optimization for different types of tissue and T cell receptor (TCR) specificities. It is strongly recommended that for each application and each TCR-specificity, the concentration of Dextramer is titrated carefully to determine the best working concentration.
This protocol has been designed for staining with PE-labeled Dextramers together with a Cy3-amplification visualization system. Alternative fluorochromes fitting your fluorescent microscope should also work. The protocol has been optimized for staining human skin tissue sections using the below listed additional reagents, however other suitable alternatives may work too.

Reagents and buffers required:
MHC Dextramer/PE (Immudex)
Normal human serum (NHS) (Sanquin)
Normal goat serum (NGS) (Sanquin)
Rabbit anti-PE antibody (Biogenesis, Poole, United Kingdom)
Goat anti-rabbit/Cy3 antibody ($F(ab)_2$, Jackson Immuno Research Laboratories,West Grove,PA)
Mouse serum (Sanquin)
Anti-human CD8/FITC antibody (Novocastra) (Leica Microsystems BV Rijswijk, The Netherlands)
VECTASHIELD mounting medium (H-1000) (Vector Laboratories, via Reactolab SA, Servion, Switzerland)
Acetone
PBS, pH7.4
BSA

Staining protocol:

1. Equilibrate the cryo-frozen tissue at -20ºC in a cryostat. Cut 6 µm sections and dry the sections on slides for 2-3 hours with fan at room temperature.

2. Fixate with acetone for 10 min.

3. Wash 3x in an adequate volume of PBS (Slides must be submerged).

4. Pre-incubate with 100 µl PBS with 5% NGS for 30 min. DO NOT wash after pre-incubation.

5. For each application the MHC Dextramer is diluted in series e.g. 1, 1/2, 1/4, 1/8 in PBS with 0.5% v/v NHS to a total volume of 100 µl for each dilution.

6. Stain the slides with 100 µl dilution each, and incubate at 4ºC ON.

7. Wash 3x in PBS.
8. Incubate with 100 µl rabbit-anti-PE antibody (1:200 dilution in PBS/1%BSA) for 30 min.
9. Wash 3x in PBS.
10. Incubate with 100 µl goat-anti-rabbit/Cy3 antibody (1:400 dilution in PBS/1%BSA) for 30 min.
11. Wash 3x in PBS.
12. Incubate with 100 µl anti-CD8/FITC antibody (1:20 dilution in PBS/1%BSA) for 1 hour.
13. Wash 3x in PBS.
14. Cover slide with VECTASHIELD mounting medium and keep at 4ºC until analysis.
15. Analyze stained slides by confocal laser scanning microscopy (CLSM).

Reference:

Kim Y, Faaij CMJM, van Halteren AGS, Schrama E, de Jong TAM, Scholler J, Egeler RM, Pavel S, Vyth-Dreese FA, van Tol MJD, Goulmy E, Spierings E. 2012. **In situ detection of HY-specific T cells in acute graft-versus-host disease-affected male skin after sex-mismatched stem cell transplantation**. *Biology of Blood and Marrow Transplantation: Journal of the American Society for Blood and Marrow Transplantation* 18(3):381-7.