

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

Chromogenic detection

Guideline for *in situ* staining of acetone-fixed cryosections.

1. Equilibrate the cryo-frozen tissue to -20°C in the cryostat. Cut 5µm sections and then dry sections on slides at room temperature. Store sections frozen until use, at -20°C
2. Equilibrate frozen sections to room temperature. Fix with acetone for 5 minutes
3. Immediately after fixation transfer slides to TBS buffer (50 mM Tris-HCL pH 7.6; 150 mM NaCl) and incubate 10 minutes at room temperature
4. Incubate with FITC-conjugated Dextramer at appropriate dilution in TBS for 30 minutes at room temperature
5. Decant, and gently tap slides against filter paper, submerge in TBS
6. Decant, add TBS and wash for 10 minutes, decant
7. Incubate with rabbit polyclonal anti-FITC antibody (e.g. Dako P5100) at 1:100 dilution in TBS for 30 minutes at room temperature
8. Repeat steps 5 and 6
9. Incubate with anti-FITC HRP-conjugated antibody (e.g. Envision anti-Rabbit HRP; Dako K4003) for 30 minutes at room temperature
10. Repeat steps 5 and 6
11. Develop with a DAB solution (e.g. DAB+; Dako K3468) for 10 minutes in fume hood
12. Rinse slides in tap-water for 5 minutes
13. Counterstain with hematoxylin (e.g. Dako S3309) for 2 minutes
14. Rinse slides in tap-water for 5 minutes
15. Mount slides

It is strongly recommended that for each application and for each specificity, the amount of Dextramer is titrated and the three different avidities are tested, in order to get optimal results. The optimal amount of Dextramer reagent may vary more than ten-fold among applications.