

Rat anti-CD31, anti-CD45 on Frozen sections (mouse)

Date: _____

Goal: To detect CD31 and CD45 on frozen sections of **mouse** tissue

Materials: Washing Buffer: 1XTBST Diluting Buffer: 1% BSA in TBST

1. Bovine Serum Albumin: Sigma Catalog #: A 4503
2. 30% H₂O₂: Fisher H325-100
3. Avidin-Biotin blocking kit: Vector: SP2001
4. Rat anti-CD31: BD Cat# 553370
5. Rat IgG: BD Cat# 553927
6. Rat anti-CD45: BD Cat# 550539
7. Biotinylated donkey anti-Rat: BD Cat#554014
8. HRP Streptavidin: Jackson labs 016-030-084
9. Mayer's hematoxylin: Sigma MHS32
10. AEC substrate: Vector SK4200
11. Aqueous mounting media: Aquamount: Vector H5501

Methods: After air-dry for 30 minutes, fix briefly in 95% alcohol and rehydrate in TBST buffer
Blocking:

1. **Block Endogenous Peroxidase:** Fisher- H325-100 Opd:
 - a. 0.3% H₂O₂ in PBST (2500µL of 30% H₂O₂ in 250mL of 1X TBST) – 30 min.
 - b. 3x Buffer Rinses PBST
2. **Block Endogenous Biotin:** Vector- SP2001 Exp:
 - a. 0.1% Avidin – 15 min. → buffer rinses – 3x
 - b. 0.01% Biotin – 15 min. → buffer rinses – 3x
 - c. Block endogenous non-specific collagen binding sites: 1% BSA/TBST, tap off

3. **Primary Reagents (separate sets):**

Dilutions:

Incubation Time:

1% BSA/TBST		Overnight at 4 degrees or 60 min @ RT <i>Always in covered humid chamber</i>
Rat IgG at 1mg/mL BD Cat# 553927		
Rat anti-CD31 BD Cat# 553370		
Rat anti CD45 BD Cat# 550539		3x Buffer Rinses TBST

4. **Secondary Reagent:**

Biotinylated anti-Rat BD Cat# 554014	1:100	30 min.
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- a. 3x Buffer Rinses TBST

5. **Tertiary Reagent:**

HRP Streptavidin Jackson 016-030-084	1:500	30 min.
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- a. 3x Buffer Rinses TBST

6. If using HRP, Chromagen: freshly made AEC Vector- SK 4200 Exp:

- a. Overlay on slides----Approximate Incubation Time: 5-15 min.
- b. Watch to see if negative control starts turning red color.
- c. If no color in negative control, for AEC, let slides go from 20 – 40 min. 3x Buffer rinse

7. Counterstain: Mayer's Hematoxylin Sigma-Aldrich MHS32

- a. Incubation Time: 1 min. followed by 3x Buffer Rinses TBST
- b. Mount and coverslip using Aqueous Mounting Media

8. Digital photomicrography to record results