

IHC Protocol on Frozen sections of mouse organs using Anti- F4/80, anti-B220 & anti-CD3

Date: **11/21/08**

Goal: Detect macrophages, B cells and T cells within frozen sections of subcutaneous tumors from mice.

Control: frozen WT mouse spleen

Washing Buffer: 1X PBS Diluting Buffer: 1% BSA in PBS Total # of slides: 16

1. Air Dry - Overnight

Fixation:

2. **Acetone** – 10 min. Cat. #: **A16P-4 (Fisher)**
 o Immediate 3x Buffer Rinses PBS

Blocking:

3. Endogenous Peroxidase Blocking: Cat. #: **H325-100 (Fisher)** Date: **Opd 10/28/08**
 o **0.03% H₂O₂ in PBS** – 30 min.
 o 3x Buffer Rinses PBS

4. Endogenous Biotin Blocking: Cat. #: **SP2001 (Vector)** Date: **Exp 7/31/10**
 o 0.1% **Avidin** – 15 min. → → → 3x Buffer Rinses PBS
 o 0.01% **Biotin** – 15 min. → → → 3x Buffer Rinses PBS

5. Endogenous Collagen Blocking:
 o **1% BSA/PBS** – 30 min. Cat. #: **A1503-506 (Sigma)**
 o **No Buffer Rinses!**

6. Primary Reagents: Dilutions: Incubation Time:

1	1% BSA/PBS [neg. control]	prediluted	2 Hrs.
2	Biotinylated anti-F4/80 (macrophages) Cat.#: MCA497B Date:	1:50	2 Hrs.
3	Biotinylated anti-B220 (Rat ant. Mo B cell) Cat.#: 553086 (Dako) Date: Opd 8/21/08	1:200	2 Hrs.
4	Biotinylated anti-CD3 (Rat ant. Mo T cell) Cat #: 553059 (Dako) Date: Opd 8/25/08	1:100	2 Hrs.

 o 3x Buffer Rinses PBS

7. Secondary Reagent: Dilutions: Incubation Time:

HRP Streptavidin Cat. #: 016-030-08 (Jackson) Date: Opd 6/17/08	1:500	30 min.
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 o 3x Buffer Rinses PBS

8. Chromagen: **AEC** Incubation Time: **10* min.** (*monitor BSA slide)
 Cat. #: **SK4200 (Vector)** Date: **Exp 8/8/09**

 o 3x Buffer Rinses PBS

9. Counterstain: **Mayer's Hematoxylin** Incubation Time: **1-2 min.**

 o 3x Buffer Rinses PBS

10. Mount in **Aqueous Gel Vectamount** Cat#: **H-5501 (Vector)**

Results, Future Plans, Notes: **Spleen Controls worked properly and showed expected antibody staining.**