

IHC Protocol on Paraffin sections of mouse organs using Biotinylated Rat Anti-Mouse F4/80

Customer:

Date:

Goal: Detect macrophages within paraffin sections of organs from mice.

Control: **paraffin sections of WT mouse spleen**

Washing Buffer: 1X PBS

Diluting Buffer: 1% BSA in PBS

Total # of slides:

Deparaffinization:

1. **Xylene 10 min. - 3x**
 - Tap off excess liquid
2. **100% Ethanol – 5 min. - 2x**
 - Tap off excess liquid
3. **95% Ethanol – 5 min. - 2x**
 - Tap off excess liquid
4. **70% Ethanol – 5 min.**
 - 3x Buffer Rinses PBS

Blocking:

5. Endogenous Peroxidase: Cat. #: **H325-100 (Fisher)** Date: **Opd**
 - **0.3% H₂O₂ in PBS – 20 min.**
 - 3x Buffer Rinses PBS
6. Endogenous Biotin: Cat. #: **SP2001 (Vector)** Date: **Exp**
 - **0.1% Avidin – 15 min.** → buffer rinses – 3x
 - **0.01% Biotin – 15 min.** → buffer rinses – 3x
 - 1% BSA/PBS
7. **Proteinase K (7 min. only) [prediluted] DAKO Cat #S3020**
 - 3x Buffer Rinses PBS
8. Endogenous Collagen Binding: Cat. #: **A1503-506 (Sigma)**
 - **1% BSA/PBS – 1 min.**
 - **Tap off. No Buffer Rinses!**
9. Primary Reagents: Dilutions: Incubation Time:

1	1% BSA/PBS [neg. control]	prediluted	Overnight @ 4°C
2	Biotinylated Rat anti-Mouse F4/80 (macrophages) Cat.#: MCA497B (Serotec) Date:	1:50	

- 3x Buffer Rinses PBS

10. Secondary Reagent: Dilutions: Incubation Time:

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

11. Chromagen: **AEC SK4200 (Vector)** Incubation Time: **10* min. (*monitor BSA)**
Cat. #: Date: **Exp**

- 3x Buffer Rinses PBS

12. Counterstain: **Mayer's Hematoxylin** Sigma HT 1079 Incubation Time: **1-2 min.**

- 3x Buffer Rinses PBS

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

Results, Future Plans, Notes:

Completed by:

Time: