

# Detection of TNF Alpha in Frozen Mouse Tissue

## Reagent and Antibody Information

[Rapid Fixx](#)

[1X Wash Buffer](#)

[0.3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[Normal Goat IgG – Affinity Purified](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Blocking Serum: Normal Donkey Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 017-000-011

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Goat Polyclonal Anti-Mouse TNF Alpha Antibody

Antigenix America

Huntington Station, NY 11746

[www.antigenix.com](http://www.antigenix.com)

1-800-558-1008

Catalog # RMF326

Secondary Antibody: Donkey Anti-Goat IgG (H+L) Biotin-SP-Conjugated

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

[www.jacksonimmuno.com](http://www.jacksonimmuno.com)

1-800-367-5296

Catalog # 705-065-147

Label Complex: R.T.U. Vectastain Elite ABC Reagent

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # PK-7100

## **Staining Procedure**

Positive Control Tissue: Thymus or LPS liver

Stain Localization: Cytoplasmic

1. Cut each frozen section at 6µm and mount on a positively charged slide.  
Immediately fix the section in Rapid Fixx solution for 7 seconds.  
Rinse the slide thoroughly in tap water to remove excess fixative, and then place it in 1X wash buffer.  
Once all the slides have undergone this process, proceed to step 2.
2. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
3. Quench endogenous peroxidase by placing slides in 0.3% hydrogen peroxide for 30 minutes.
3. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
4. Heat-Induced Epitope Retrieval Using The Steamer  
Add distilled water to the bottom portion of the steamer.  
Preheat 200ml of 1X citrate buffer in a Tissue Tek® container in the steamer between 95°C and 100°C.  
Immerse a full rack of slides into the citrate buffer and place the lid back on the steamer.  
(Insert blank slides into any empty slots in the rack to ensure even heating of slides)  
Steam the slides for 20 minutes.  
Remove container from steamer and cool for 20 minutes.  
Rinse the slides in 2 changes of distilled water for 3 minutes each time.
5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each time.
6. Block with 5% normal donkey serum for 20 minutes at room temperature.  
Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no  
Apply avidin block for 15 minutes at room temperature.  
Quick rinse in 1X wash buffer.  
Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.  
ONLY WIPE EXCESS BLOCK.

8. Apply primary antibody at a 1:10 dilution. Incubate overnight at 4°C.  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

For negative control slides, dilute normal goat IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:10 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate overnight at 4°C.

Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_

\*\*\*\*\*Next Day\*\*\*\*\*

9. Bring the slides up to room temperature in 1X wash buffer for at least 15 minutes.
10. Apply the donkey anti-goat secondary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.  
Lot # \_\_\_\_\_ Date Reconstituted \_\_\_\_\_
11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
12. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.  
Exp. Date \_\_\_\_\_ New Kit: yes / no
13. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
14. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.  
(Add 1 drop of DAB per ml of substrate)  
Lot # \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no
15. Rinse the slides in tap water 3 minutes.
16. Counterstain with hematoxylin for 20 seconds.
17. Rinse the slides in tap water until water is clear.
18. Gently agitate slides in 1X wash buffer until the tissues turn blue.
19. Dehydrate through the following solutions:

| Solutions    | Repetitions | Time      |
|--------------|-------------|-----------|
| 95% Ethanol  | 1 time      | 3 minutes |
| 100% Ethanol | 3 times     | 3 minutes |
| Xylene       | 2 times     | 5 minutes |

20. Coverslip

*Updated 08/08/03*