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IMMUNOFLUORESCENCE STAINING HANDBOOK

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INTRODUCTION

This immunofluorescence staining kit (IFK) is designed to determine the localization of target antigens present within frozen and paraffin-embedded tissue preparations for approximately 100 to 150 slides. Briefly, an antigen specific primary antibody is applied to the tissue section slide whereby the primary antibody binds to the target antigen. This is followed by incubation with a fluorescein-isothiocynate (FITC) labeled secondary antibody, which recognizes the primary antibody. Localization of the antigen/antibody/secondary antibody complex can be observed via darkfield microscopy (with filters appropriate for fluorescein-isothiocynate detection). The staining pattern visible in darkfield microscopy will show a green to yellow-green emission.

Although this kit can be used for paraffin-embedded tissues, we recommend that frozen tissue sections be used, because they are better in preserving antigenic content. For paraffin-embedded tissue sections, de-waxing and re-hydration steps are required, followed by an antigen-retrieval step as described herein. Detailed protocols on the preparation of frozen and paraffin-embedded sections for immunofluorescence staining can also be found in any good immunostaining textbook.

KIT COMPONENTS

	Component	Amount
1	Primary antibody (lyophilized powder)	dissolve into 50 ul
2	FITC-labeled secondary antibody	100 ul
3	Normal rabbit or guinea pig serum (lyophilized powder)	dissolve into 50 ul
4	5X phosphate buffered saline, pH 7.4 with 0.1% sodium azide	10 ml
5	3% triton X-100/water	5 ml
6	Glycerin-PBS (9:1)	2 ml
7	Normal goat serum	dissolve into 13 ml

MATERIALS NOT PROVIDED

1. Xylene (for deparaffinization)
2. Ethanol (for sample rehydration)
3. Distilled water
4. PBS buffer (0.1M phosphate buffered saline) pH 7.4
5. Antigen retrieval solution: citrate buffer (0.01M), pH 6.0.
6. Darkfield microscope (transmitted or reflected light) with filters appropriate for fluorescein-isothiocynate detection (excitation 440-490nm; emission 520-560nm).
7. Sample tissue slides

IMPORTANT NOTES

1. Storage: Keep at 4°C upon arrival and during use. Aliquot and store at -20°C for long-term storage. Avoid repeated freeze/thaw cycles. When stored properly, the expected shelf life is 1 year.

2. Tissue fixation: 4% paraformaldehyde in 0.1M PBS, pH 7.4.

3. Do all of the steps of the staining procedure at room temperature, except the incubation with primary antibody overnight at 4°C.

4. Do not allow tissue slides to dry. Incubation of slides should be in a moist chamber.

5. Optimal dilution and incubation time of primary antibody may be adjusted depending on the preparation of the tissue sample. We recommend that each laboratory determine an optimum working dilution for its particular sample. The remaining reconstituted primary antibody serum should be stored at 4°C for future use (within three months). For longer term storage, store at -20°C and avoid frequent freeze-thaws.

6. Store developed slides in the dark to prevent fluorescence fading.

PREPARATION OF REAGENTS

1) **0.1M PBS containing 0.3% Triton X-100:** Dilute the 10 ml of 5X PBS concentrate with 35 ml of distilled water. Add 5 ml of 3% Triton X-100/water to the PBS solution.

2) **Primary antibody working solution:**

a. Reconstitute the lyophilized primary antibody with 50 ul of PBS buffer pH 7.4.

b. Due to inherent differences in tissue preparation, a dilution series of 1:250, 1:1000, 1:2000, 1:4000, 1:8000 and 1:16000 in Normal Goat Serum Blocking Solution is strongly recommended to ascertain the optimum antibody dilution, particularly for the initial experiment.

3) **Blocking buffer:** Rehydrate NGS with 13 ml of 0.1M PBS containing 0.3% Triton X-100.

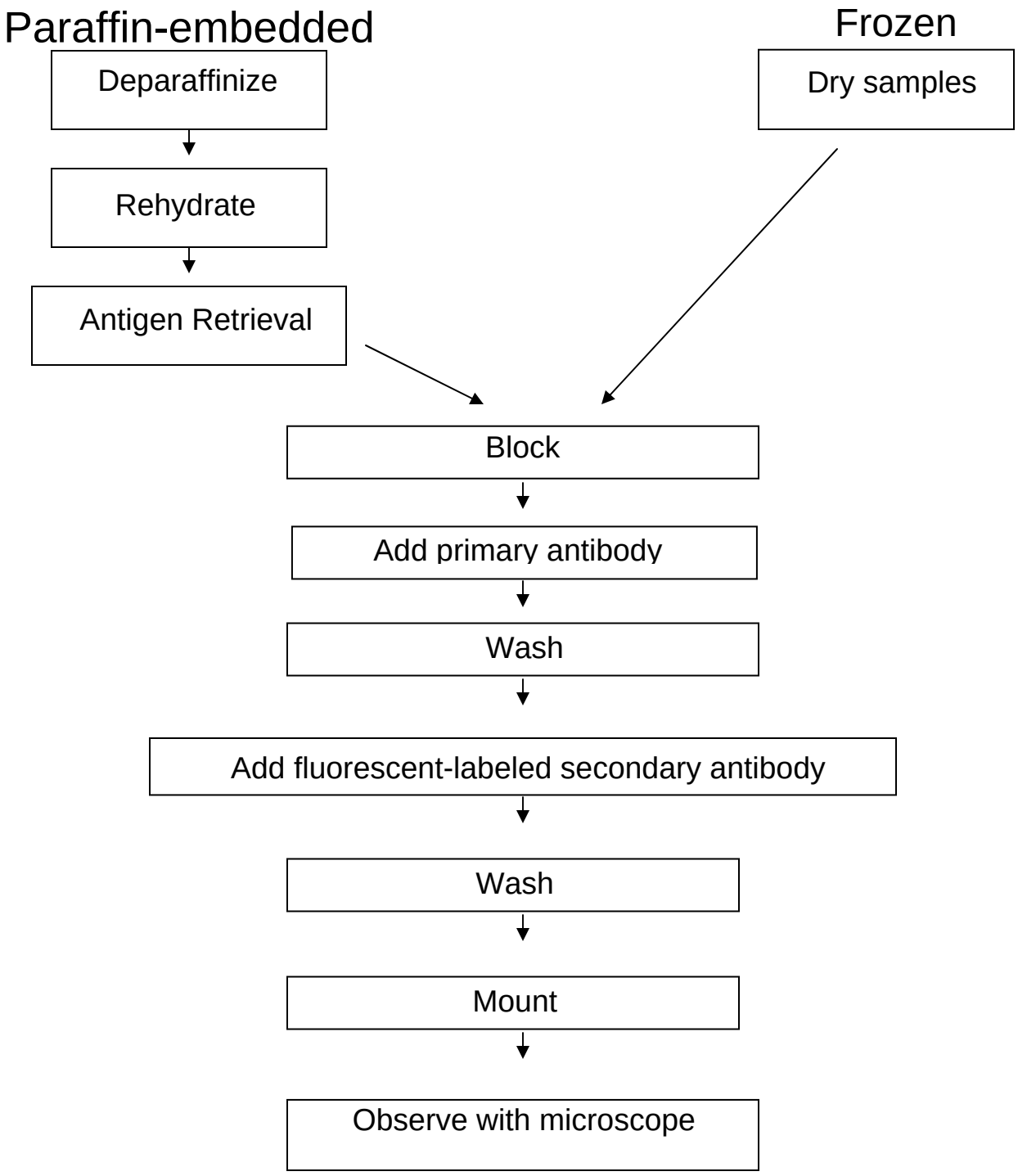
4) **Negative control serum:**

a. Rehydrate the negative control serum with 50 ul of 0.1M PBS, pH 7.4.

b. Dilute serum 1:250 in normal goat serum blocking solution.

5) **Fluorescent-labeled secondary antibody:** Dilute the fluorescent conjugated secondary antibody with 9.9 ml of 0.1M PBS containing 0.3% Triton X-100. This will yield 10 ml of labeled secondary antibody (1:100).

STAINING PROCEDURE OVERVIEW



This summary is not intended to replace the preparation steps, precautions and procedures shown in this booklet. Please consult those sections for complete directions.

STAINING PROCEDURE

Please read "IMPORTANT NOTES" on pages 5 and "PREPARATION OF REAGENTS" on page 6 before starting.

1. **Prepare sample tissue slides.**
   - **a. For <u>paraffin-embedded</u> tissue slides**:
     - **i. De-wax with xylene.**
     - **ii. Re-hydrate in a graded ethanol series.**
     - **iii. Rinse for 5 minutes in tap water.**
     - **iv. Perform antigen retrieval (recommended)**
       i. Place slides in a Coplin jar or plastic slide container.
       ii. Fill with antigen retrieval buffer (0.01M citrate buffer, pH 6.0).
       iii. Place container in a steamer (rice cooker) with 150 ml of water.
       iv. Press “cook” button.
       v. After it is done 10 to 20 minutes, remove container from cooker and open lid.
       vi. Allow to cool for at least 20 minutes.
   - **b. For <u>frozen tissue</u> slides:**
     - **i. Thaw frozen tissue sample.** Expose the frozen tissue sections to room temperature and thaw for 5 minutes.

2. **Wash slides** 3 times for 10 minutes each with 0.01M phosphate buffered saline. Blot slides but do not touch the tissue or allow it to dry.

3. **Block slides.** Apply 200 ul of NGS (1:10) on the tissue sections. Incubate the slides at room temperature for 30 minutes in a closed incubation chamber. Blot slides but be careful not to touch the tissue or allow it to dry.

4. **Incubate slides with primary antibody.** Apply approximately 2 drops (100 ul) of primary antiserum (1:250) or negative control on

tissue sections. Incubate the slides at 4^{o}C for 24 hours in the same chamber.

5. **Wash slides** 3 times for 10 minutes each with 0.01M Phosphate buffered saline. Blot slides but do not touch the tissue or allow it to dry.

6. **Incubate slides in labeled secondary antibody.** Apply 200 ul of secondary antibody (fluorescent conjugated goat anti-rabbit or anti-guinea pig IgG) (1:100). Incubate the slides at room temperature for 60 minutes in the same chamber.

7. **Wash the slides** 3 times for 10 minutes each with 0.01 M PBS. Blot the excess PBS from the slides with an absorbent wipe. Do not touch the tissue or allow it to dry.

8. **Mount** with Glycerin-PBS (9:1) and affix coverslip.

9. **Observe** the stained tissue with a darkfield microscope for incident light fluorescence.

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