



Immunofluorescence Detection kits

For detection of mouse, rabbit, goat, rat, sheep, human, guinea pig, and chicken primary antibodies

Size: 500 Tests

- Catalog #: FK-011, Mouse Kit**
- Catalog #: FK-022, Rabbit Kit**
- Catalog #: FK-033, Goat Kit**
- Catalog #: FK-044, Rat Kit**
- Catalog #: FK-055, Sheep Kit**
- Catalog #: FK-066, Chicken Kit**
- Catalog #: FK-077, Guinea Pig Kit**
- Catalog #: FK-088, Human Kit**

Manufactured by

Zyagen

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Ordering Information

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Technical Information

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Intended use: Zyagen Immunofluorescence detection kits are for Research Use Only. Kits are not recommended for diagnostic or therapeutic procedures use under any circumstances.

Introduction: Immunofluorescence (IF) is a powerful technique widely used for detection of antigens in histological and cytological specimens. The basic principle of Immunofluorescence is the use of fluorescent dye-linked antibodies to detect tissue antigens. Positive staining will show an emission of fluorescent light in a dark field on the slide at the site of the antigen localization. In Zyagen Immunofluorescence detection kits, fluorescein isothiocyanate (FITC) is used as a fluorescent dye for visualization of antigenic structures in the tissues. FITC emits a green to yellow light. The high sensitivity and specificity of Zyagen Immunofluorescence detection kits is achieved by using non specific IgGs-absorbed secondary biotinylated antibodies and high sensitive streptavidin conjugated to FITC. Kits work on frozen and paraffin slide tissue sections, cytological specimens, and free-floating tissue sections.

Warning and Precautions:

- Do not interchange reagents of this kit with components from any other Zyagen or other vendor detection kits.
- Any changes in the kit staining procedures (dilution, washing, incubation time or temperature) can alter the performance.
- Warm-up the reagents to room temperature before adding to the sections.
- Make solutions shortly before use and discard the leftover.

Experimental Controls:

Positive controls:

- To be sure that the staining system is working properly; include tissue sections that are known containing the antigen of interest as tested by other staining systems.

Negative controls:

- Substitute buffer free of serum for the primary antibody. Some normal sera containing unknown antibodies are not recommended for use as negative controls.
- Test control samples with non-immune immunoglobulin (pre-immune serum) of the same isotope of the primary antibody. Use the non-immune control if the primary antibody is monoclonal.
- Test control samples with neutralized primary antibody (preincubated with corresponding immunogen). Use the neutralized antibody control if the primary antibody was raised against peptide.
- Test control samples with the primary antibody absorbed by immobilized protein. Use the absorbed antibody control if the primary antibody was raised against protein.

Storage: Store Kit at 2-8 °C. Some of the kit components should be stored at -20 °C.

Kit Components:

Reagent 1: Serum Blocking Reagent (3 ml), store at 4°C

Reagent 2: Primary Antibody Diluent reagent -Component contains BSA (3ml), store at 4°C

Reagent 3: Biotinylated Secondary Antibody (whole H&L chains IgG) (0.125ml), store at -20°C (solution will not freeze).

Reagent 4: High Sensitive Streptavidin-FITC Conjugate (HSS-FITC) (0.25ml), store at -20°C (solution will not freeze).

Reagent 5: Counter Staining-DAPI (optional) (0.1ml), store at -20°C

Reagent 6: Aqueous Anti-Fade Fluorescent Mounting Medium (25ml), store at 4°C

Reagents required but not provided:

- Primary Antibody: Consult the manufacturer/vendor for suitability of the primary antibody for immunohistochemistry and follow their instruction for the optimal working concentration and incubation time.
- Washing Buffers: Phosphate Buffered Saline pH 7.4 (PBS; Zyagen part number # 170-110).
- Antigen retrieval solutions for paraffin sections: use any Heat Induced Epitope Retrieval solutions (Zyagen part number # 170-111). You can also use any unmasking digestive enzymes such as trypsin, pepsin, chymotrypsin, or Pronase to expose the antigen.
- Distilled or deionized water.
- Xylene for deparaffinization of paraffin sections.
- Absolute Ethanol for rehydration and dehydration of tissue sections.

Preparation of working concentration:

To promote activity and performance, kit components are provided in concentrated formats; it takes less than one minute to make each solution. Dilute reagents to working concentrations shortly before use and discard the leftover diluted solutions.

Calculate the total volume of each solution needed for processing each slide. Note: 100µl of each working solution is required to cover a small tissue section ($\approx 0.5\text{cm}^2$) and 200µl to cover large section ($\approx 1\text{cm}^2$) on a single slide. For processing 20 slides with 100µl each you need 2 ml of each solution.

- Serum Blocking Solution: add 0.1ml (or 2 drops) of reagent #1 to 1.9ml PBS and vortex for few seconds.
- Primary Antibody Diluent Solution: add 0.1 ml (or 2 drops) of reagent #2 to 1.9ml PBS and vortex for few seconds.
- Biotinylated Secondary Antibody Solution: add 4 µl of reagent #3 to 2ml PBS and vortex for few seconds.
- Streptavidin-FITC Conjugate Solution: add 8µl of reagent #4 to 2ml PBS and vortex for few seconds.
- Counter Staining DAPI (Optional), add 2µl of reagent # 5 in 2ml PBS.
- Aqueous Anti-Fade fluorescent mounting medium (reagent# 6), Ready to use.

Preparation of tissue sections:

Frozen sections:

- Fix tissue sections for 10 minutes in cold acetone or any other suitable fixatives such as ethanol, 10% neutral buffered formalin, or 4% paraformaldehyde.
- After fixation with acetone or ethanol, air dry slides at room temperature for at least 20 minutes. There is no need for air drying if you are using formalin-based fixatives.
- Proceed with staining procedure.

Paraffin Sections:

- Deparaffinize sections with xylene or any other clearing agents (at least 2 changes, 5 minutes each)
- Rehydrate sections through graded ethanol solutions (2x100%, 80%, and 50%) to water, 3 minutes in each solution.
- Retrieve antigen by Heat Induced Epitope Retrieval solution using any recommended protocol. We are routinely using citrate buffer following this protocol:
 - Add adequate amount of 0.1M citrate buffer pH 6.0 (Zyagen part number # 170-111) to a coplin jar or staining dish and heat to 99°C/100°C in a water bath. To speed the process, heat citrate buffer to boiling temperature by microwave and then transfer jar/staining dish to the water bath.
 - Place slides in a slide holder in the heated citrate buffer and incubate for 15 minutes.
 - Remove jar/dish from the water bath and allow slides to cool down to room temperature (30-40 minutes) inside the buffer.
 - Rinse for 3 minutes in water.

For antigen retrieval by enzymatic digestion, follow instructions provided by the enzymes supplier.

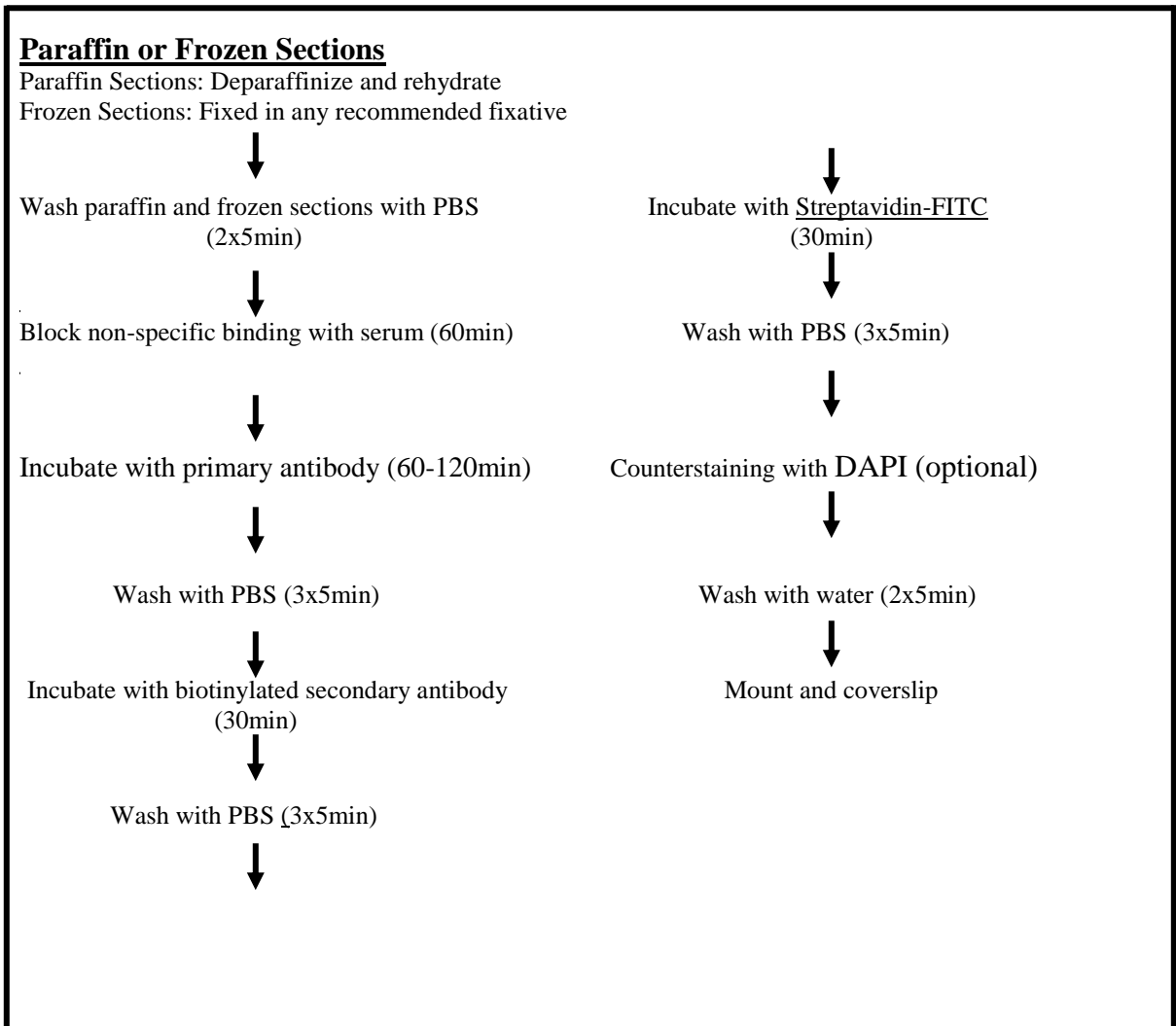
- Proceed with staining procedure

Staining Procedure:

- Wash slides 2 times with washing buffer (PBS; Zyagen part number # 170-110), 5 minutes each.
- Cover sections with 100-200µl of serum blocking solution depending on the section size and incubate at room temperature for 60 minutes in a humidified chamber.
- Drain slides and wipe off excess serum solution. Do not wash.
- Cover sections with 100-200µl of primary antibody diluted in the Antibody Diluent Solution and incubate for 1-2 hour at room temperature or O/N at 4°C. Longer incubation time may be required. It is highly recommended to follow instruction of the antibody supplier for the working concentration and incubation time.
- Wash 3 times with washing buffer, 5 minutes each.
- Drain slides and wipe off excess buffer.
- Cover sections with 100-200µl of biotinylated secondary antibody solution and incubate for 30 minutes at room temperature.
- Wash 3 times with washing buffer, 5 minutes each.
- Drain slides and wipe off excess buffer.

- Cover sections with 100-200µl of streptavidin-FITC conjugate solution and incubate for 30 minutes at room temperature.
- Wash 3 times with washing buffer, 5 minutes each.
- If desired, cover sections for 1-2 minute with DAPI solution.
- Wash 3 times with washing buffer, 5 minutes each
- Cover with Anti-fade fluorescent mounting medium, coverslip, and seal with a nail polish.
- Store in a slide container at 4°C.

Summary of Staining Procedure:



Troubleshooting:

Problem	Possible cause	Suggestions
Weak or no staining (poor signal)	Primary antibody concentration and/or incubation time is too low	Increase antibody concentration and/or incubation time
	Primary antibody is not suitable for immunohistochemistry or type of tissue sections. Some antibodies work on paraffin but not on frozen sections and vice versa.	Use antibody recommended for IHC and tissue sections under study.
	Inappropriate fixative or incomplete fixation	Optimize fixation time and select fixative recommended for the tissue sections under study
	Incomplete deparaffinization	Increase deparaffinization time. Use fresh solvent
	Incomplete antigen retrieval procedures/permeabilization of the tissue sections.	Optimize antigen retrieval procedures.
	Kit reagents had past their expiration date	Use new kit
High background (Poor Signal: Noise Ratio)	Inadequate washing	Increase washing steps or times
	Primary antibody concentration and/or incubation time is too high	Decrease antibody concentration and/or incubation time
	Non-specific binding	Increase the incubation time with serum blocking reagents
	Tissue sections were dried during immunostaining	Work quickly to avoid drying of sections. Discard dried slides.
	High tissue content of auto-fluorescence particularly in paraffin sections	Auto-fluorescence in tissue sections is mainly induced by fixatives. Before blocking step with serum, treat sections with 1 mg/ml (0.1%) sodium borohydride in PBS 2times, 5-10 min each. Then wash with PBS 2-3 times to remove sodium borohydride.
	High tissue content of endogenous avidin and/or biotin	Incubate sections for at least 15 min each with avidin and biotin quenching solutions (Avidin/Biotin Blocking kit; Zyagen part number # 170-112)
Sections detached from slides	Glass slides are not suitable for IHC	Use charged slides or poly-l-lysine-treated slides
	Excessive washing	Reduce washing steps or times. Avoid excessive shaking of slides during washing
Deterioration of Tissue sections morphology	Incomplete fixation	Increase fixation time. Use fresh fixative
	Overdigestion with permeabilization agents	Decrease concentration and/or incubation time with permeabilization agents

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