Peroxidase Immunohistochemistry Detection kits

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

Size: 500 Tests

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

Manufactured by

Zyagen
10225 Barnes Canyon Road
San Diego, CA 92121, USA

Ordering Information

For ordering information, please visit our website at **www.zyagen.com** or contact Customer Service Representatives at:

Tel: 1 888 425 7095 (Toll Free)
1 858 546 0720
Fax: 1 858 546 0236
E-mail: zcs@zyagen.com

Technical Information

For any technical information or assistance with procedures, please contact our Technical Service Representative at:

Tel: 1 888 425 7095 (Toll free)
1 858 546 0720
E-mail: zinfo@zyagen.com

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

Introduction: Immunohistochemistry (IHC) is a powerful technique widely used for detection of antigens in histological and cytological specimens. The basic principle of IHC is the use of enzyme-linked antibodies to detect tissue antigens. The colorless substrate is converted by enzyme into a colored product that precipitates on the slide at the site of the antigen localization. In Zyagen peroxidase detection kits, Chromogen DAB (3, 3'-diaminobenzidine) is used as a substrate of horseradish peroxidase (HRP) for visualization of antigenic structures in the tissues. This substrate produces a brown color product which is insoluble in alcohol. The high sensitivity and specificity of Zyagen system immunostaining kits is achieved by using non specific IgGs-absorbed secondary biotinylated antibodies and sensitive streptavidin conjugated to HRP. 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.
- DAB Buffer is harmful. If contact with skin, wash with plenty of water. In case of contact with eyes, rinse immediately with water and seek medical advice.
- DAB chromogen (3, 3'-diaminobenzidine) is classified as a carcinogenic product. Wear gloves and protective clothing to avoid contact with skin.
- Follow federal and State regulations to dispose used DAB chromogen.

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 ºC. Some of the kit components should be stored at -20 ºC.

Kit Components:

Reagent 1: Serum Blocking Reagent (3 ml), store at 4ºC
Reagent 2: Primary Antibody Diluent reagent -Component contains BSA (3ml), store at 4oC
Reagent 3: Biotinylated Secondary Antibody (whole H&L chains IgG) (0.125ml), store at -20 ºC (solution will not freeze).
Reagent 4: Streptavidin-HRP Conjugate (HSS-HRP) (0.25ml), store at -20 ºC (solution will not freeze).
Reagent 5A: DAB Chromogen (6 ml), store at 4ºC. Keep in darkness in side the kit. Avoid prolong exposure to light
Reagent 5B: DAB Buffer (100 ml) containing 0.1% hydrogen peroxide, Store at 4ºC
Reagent 6: Counter Staining-Hematoxylin (50 ml), store at 4ºC or room temperature
Reagent 7: Non-Aqueous Mounting Medium (50 ml), store at 4ºC or room temperature
Reagent 8: Peroxidase Blocking Reagent-30%Hydrogen peroxide (6 ml), store at 4ºC

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 (≈0.5cm^2) and 200µl to cover large section (≈1cm^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 of reagent #1 to 1.9ml PBS and vortex for few seconds.

- Primary Antibody Diluent Solution: add 0.1 ml 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-HRP Conjugate Solution: add 8µl of reagent #4 to 2ml PBS and vortex for few seconds.
- DAB Substrate Solution: add 0.1 ml of DAB chromogen (reagent #5A) to 1.9ml DAB buffer (Reagent #5B) and vortex for few seconds. For maximum sensitivity add 0.15 ml chromogen to 1.85 ml DAB buffer. Use within 4 hours.
- Counter Staining-Hematoxylin (Reagent #6), Ready to use.
- Non-Aqueous Mounting Medium (Reagent# 7), Ready to use.
- Peroxidase Blocking Solution: add 0.2 ml of reagent #8 to 1.8ml PBS and vortex for few seconds.
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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 99ºC/100ºC 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 peroxidase blocking solution. Incubate paraffin sections for 5 minutes and frozen sections for 1 minute.
- Wash with washing buffer for 10 minutes, drain slides and wipe off excess buffer.
- 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 4ºC. 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-HRP conjugate 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 DAB substrate solution and incubate for up to 20 minutes. Monitor intensity of immunostaining under microscope until the desired color intensity is reached.
- Wash slides with water 2 times, 5 minutes each.
- Cover sections for 1-2 minute with hematoxylin and rinse with water. Do not overstain sections. Overstaining with hematoxylin may mask the weak immunostaining.
- Dehydrate through graded ethanol solutions (85%, 95%, and 2x 100%, 5 minutes each) and clear with xylene or any other clearing agent (at least 2 changes, 5 minutes each)
- Add 2 drops of xylene-based mounting medium using a glass rod and coverslip.
- Allow slides to dry at room temperature or on slide warmer and examine under microscope.

Summary of Staining Procedure:

Paraffin or Frozen Sections

Paraffin Sections: Deparaffinize and rehydrate
Frozen Sections: Fixed in any recommended fixative

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Wash paraffin and frozen sections with PBS (2x5min)

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Block Endogenous peroxidase activity (5 min for paraffin sections and 1 min for frozen sections)

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Wash with PBS (10min)

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Block non-specific binding with serum (60min)

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Incubate with primary antibody (60-120min)

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Wash with PBS (3x5min)

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Incubate with biotinylated secondary antibody (30min)

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Wash with PBS (3x5min)

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Incubate with Streptavidin-HRP (30min)

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Wash with PBS (3x5min)

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Incubate with substrate-chromagen mixture (3-20 min)

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Wash with water (2x5min)

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Counterstaining with Hematoxylin (optional)

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Wash with water (2x5min)

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Dehydrate, mount, and coverslip

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 understudy.
	Inappropriate fixative or incomplete fixation	Optimize fixation time and select fixative recommended for the tissue sections understudy
	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
	Presence of enzyme inhibitors such as sodium azide in the buffers/solutions	Use buffer free of sodium azide or any other inhibitors.
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 endogenous peroxidase	Increase concentration and/or incubation time of endogenous peroxidase quenching solution.
	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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