Alkaline Phosphatase Immunohistochemistry Detection kits

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

Size: 500 Tests

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

TABLE OF CONTENTS

Contents	Page
Introduction	3
Warning and Precautions	3
Experimental Controls	3
Storage	4
Kit components	4
Reagents required but not provided	4
Preparation of working concentration	4
Preparation of Tissue Sections	5
Staining Procedure	5
Summary of Staining Procedure	7
Troubleshooting	8
References	9

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 alkaline phosphatase detection kits, fast red is used as a substrate of alkaline phosphatase (AP) for visualization of antigenic structures in the tissues. This substrate produces a bright red color product. 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 alkaline phosphatase. 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.
- Do not use PBS with Alkaline Phosphatase detection kits.

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 ^{o}C. Some of the kit components should be stored at -20 ^{o}C.

Kit Components:

Reagent 1: Serum Blocking Reagent (3 ml), store at 4^{o}C
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 °C (solution will not freeze).
Reagent 4: Streptavidin-AP Conjugate (0.25ml), store at -20 °C (solution will not freeze).
Reagent 5A: Fast Red Chromogen (20 tablets), store at -20°C. Keep in darkness. Avoid prolong exposure to light.
Reagent 5B: Fast Red Buffer (100 ml), Store at 4 °C
Reagent 5C: Levamisole solution (2ml), Store at -20 °C
Reagent 6: Counter Staining-Hematoxylin (50 ml), store at 4 °C or room temperature
Reagent 7: Aqueous Mounting Medium (50 ml), store at 4 °C or room temperature

.

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.
- Buffer: Tris Buffered Saline solution (TBS), pH 7.6 [Final working concentration: 50mM Tris base and 0.9% NaCl (Zyagen part number # 170-112)]. Use TBS for washing sections and diluting kit reagents. Do not use PBS with Alkaline Phosphatase detection kits.
- 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 TBS and vortex for few seconds.
- Primary Antibody Diluent Solution: add 0.1 ml of reagent #2 to 1.9ml TBS and vortex for few seconds.
- Biotinylated Secondary Antibody Solution: add 4 µl of reagent #3 to 2ml TBS and vortex for few seconds.
- Streptavidin-AP Conjugate Solution: add 8µl of reagent #4 to 2ml TBS and vortex for few seconds.
- Fast Red Substrate Solution: add one fast red tablet (reagent #5A) to 5ml fast red buffer (Reagent #5B) and shake until fully dissolved. Apply immediately to the sections. If tissues contain endogenous alkaline phosphatase and using frozen

sections, add 100µl of levamisole solution (reagent# 5C) to the fast red substrate solution. There is no need for levamisole if you are using paraffin sections since boiling temperature during antigen retrieval processing step (see below) destroy endogenous AP.

- Counter Staining-Hematoxylin (Reagent# 6), Ready to use.
- Aqueous Mounting Medium (Reagent # 7), 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 (TBS; Zyagen part number # 170-112), 5 minutes each, 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 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-AP 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 fast red substrate solution and incubate for 5 minutes. Rinse with D1 water and cover sections again with 100-200µl of fast red substrate solution for an additional 5minutes (repeat until desired intensity is achieved).
- Wash slides with D1 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.
- Add 2 drops aqueous 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

↓

Wash paraffin and frozen sections with TBS (2x5min)

↓

Block non-specific binding with serum (60min)

↓

Incubate with primary antibody (60-120min)

↓

Wash with TBS (3x5min)

↓

Incubate with biotinylated secondary antibody (30min)

↓

Wash with TBS (3x5min)

↓

Incubate with Streptavidin-AP (30min)

↓

Wash with TBS (3x5min)

↓

Incubate with substrate-chromogen mixture

↓

Wash with D1 water (2x5min)

↓

Counterstaining with Hematoxylin (optional)

↓

Wash with water (2x5min)

↓

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 phosphate in buffers/solutions	Use buffer free of phosphate or any other inhibitors. Do not use PBS in AP detection system
High background (Poor Signal: Noise Ratio)	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 Alkaline phosphatase.	Tissues such as liver, kidney, intestine, and placenta naturally contain endogenous AP. Adding Levamisole solution to the fast red substrate buffer can reduce the endogenous AP. However, levamisole can reduce the overall positive staining and must be used with caution.
	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)
	Inadequate washing	Increase washing steps or times
Sections detached from slides	Excessive washing	Reduce washing steps or times. Avoid excessive shaking of slides during washing
	Glass slides are not suitable for IHC	Use charged slides or poly-l-lysine-treated slides
Deterioration of Tissue sections morphology	Overdigestion with permeabilization agents	Decrease concentration and/or incubation time with permeabilization agents
	Incomplete fixation	Increase fixation time. Use fresh fixative

References

1. Larsson, L.I. (1988). Immunocytochemistry: Theory and Practice. Crc Press, Boca Raton, Fl.
2. Köller, U., *Et Al.* (1986). *J. Immunol. Methods* **86**, 75.
3. Wood, G.S. And Warnke, R. (1981). *J. Histochem. Cytochem.* **29**, 1196-1204.
4. Naritoku, W.Y. And Taylor, C.R. (1982). *J. Histochem. Cytochem.* **30**, 253-260.
5. Hsu, S.M., *Et Al.* (1981). *Am. J. Clin. Path.* **75**, 816.
6. Hsu, S.M., *Et Al.* (1981). *J. Histochem. Cytochem.* **29**, 577-580.
7. Guesdon, J.L., *Et Al.* (1983). *J. Histochem. Cytochem.* **27**, 1131.
8. Adams J.C., (1981). *J. Histochem. Cytochem.* **29**, 775.
9. Bullock, G.R., and Petrusz, eds (1990) Techniques in immunohistochemistry, Vol 2: Academic Press: New York.
10. Lacey, A.J. ed (1989) Light microscope in Biology. A practical Approach, IRL Press New York
11. Cuello A.C. ed (1993) Immunohistochemistry: Method in the Neuroscience, Vol 14, John Wiley & Sons, New York.