

Immunohistochemistry Kit: **MaxTag™ Histo IHC Kit** for IHC using Rabbit Primary Antibody

Catalog # KHA003

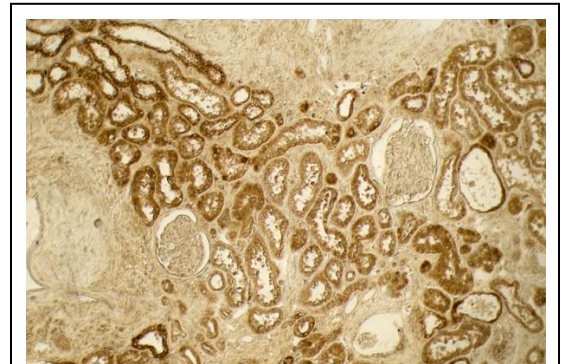
I. Overview

Rockland Immunochemicals' **MaxTag™ Histo** Immunohistochemistry Kit is intended to provide a simple, reliable and convenient detection system for immunohistochemical staining. As such, the kit can be used for the identification of specific constituents in tissue sections or immobilized cells. In order to detect the reaction site, the antibody complex is labeled with an enzyme (peroxidase) that can be reacted with a suitable substrate (DAB) to give a colored product. This kit also provides for the counter staining of nuclei to reveal tissue architecture. The stain and substrate color fade slowly, if at all, allowing the slides to be stored for future use. The contents of this kit are formulated for maximum ease of use and are color coded to eliminate confusion. A sample protocol and recommended conditions for use are provided. Formaldehyde fixation is suggested as the routine initial method of choice for tissue and cell fixation. The provided protocol is a guideline. Any procedure can be altered according to specific experimental requirements. The use of both highly purified and adsorbed antibody conjugates and matched reagents reduces any lot-to-lot variation. This kit can process 100-200 immunostains on standard microscope slides and is stable for at least 1 year when stored at +4°C.

Please read the entire product insert prior to use.

II. Kit Principle

This **MaxTag™ Histo** Immunohistochemistry Kit allows for the detection of primary **rabbit** polyclonal or monoclonal antibody provided by the user. Kit relies upon the high specificity of avidin-biotin binding for visualization of an antigen. The antigen (usually within immobilized cells or tissue) is fixed and adhered to a glass microscope slide. A primary antibody reacts with the immobilized antigen to form an antigen-antibody complex. A second biotinylated antibody derived from goat and specific for the primary antibody reacts with the complex. Streptavidin conjugated to the enzyme peroxidase reacts with this complex localizing the enzyme at the site of the antigen. Finally, substrate is added causing a reaction with the peroxidase as a colored precipitate to form on the slide at the location of the antigen. The slide is viewed through a standard light microscope and, if desired, photographed for a permanent record.



Immunohistochemistry Kit – IHC kit with DAB substrate can generate publication quality results for novices and experts alike. Expect reliable and reproducible signal with low background staining when using the **MaxTag™ Histo** IHC Kit.

III. Intended Use

Use Rockland Immunochemicals' **MaxTag™ Histo** Immunohistochemistry Kit for the detection of **rabbit** polyclonal or monoclonal antibody bound to antigens within tissue immobilized on glass microscope slides. Detect these complexes by allowing the chromogenic substrate to form color at the reaction site and visualize using a light microscope. See "Additional Notes" for helpful tips. If you require additional assistance please call or e-mail our technical service representatives at 800-656-7625 or tech@rockland-inc.com.

IV. Storage and Stability

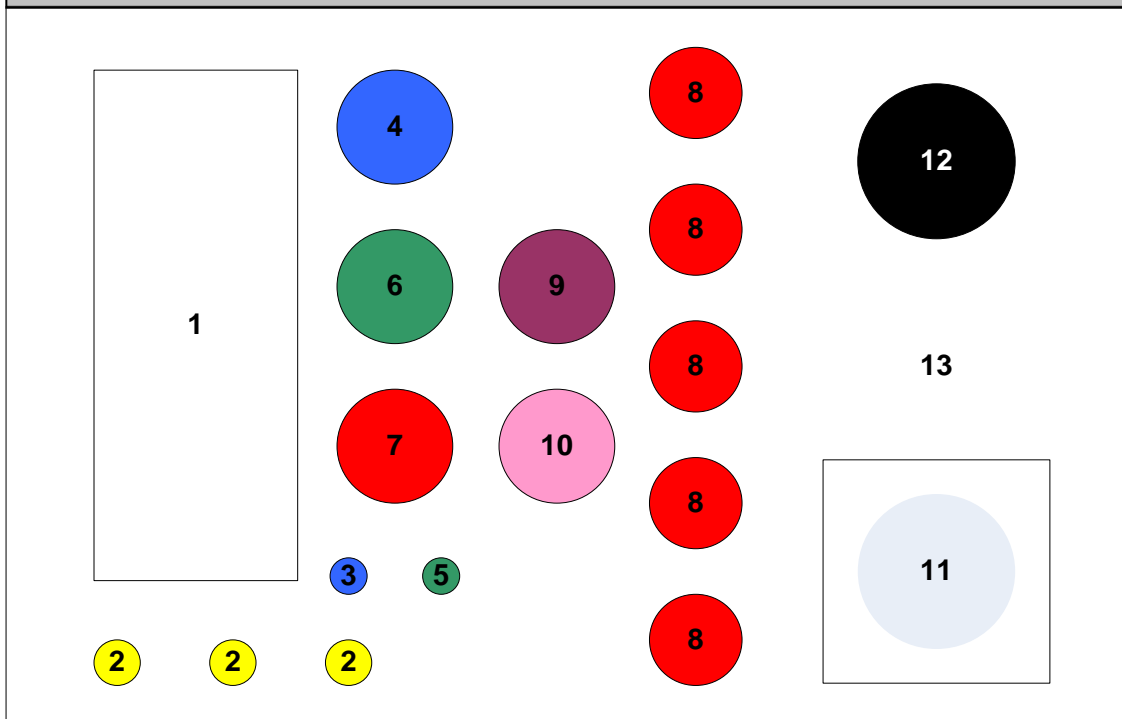
This kit is stable for at least one year when stored at +4°C. Individual components are stable for 3-4 weeks after dilution when stored at 4°C.

V. Number of Assays

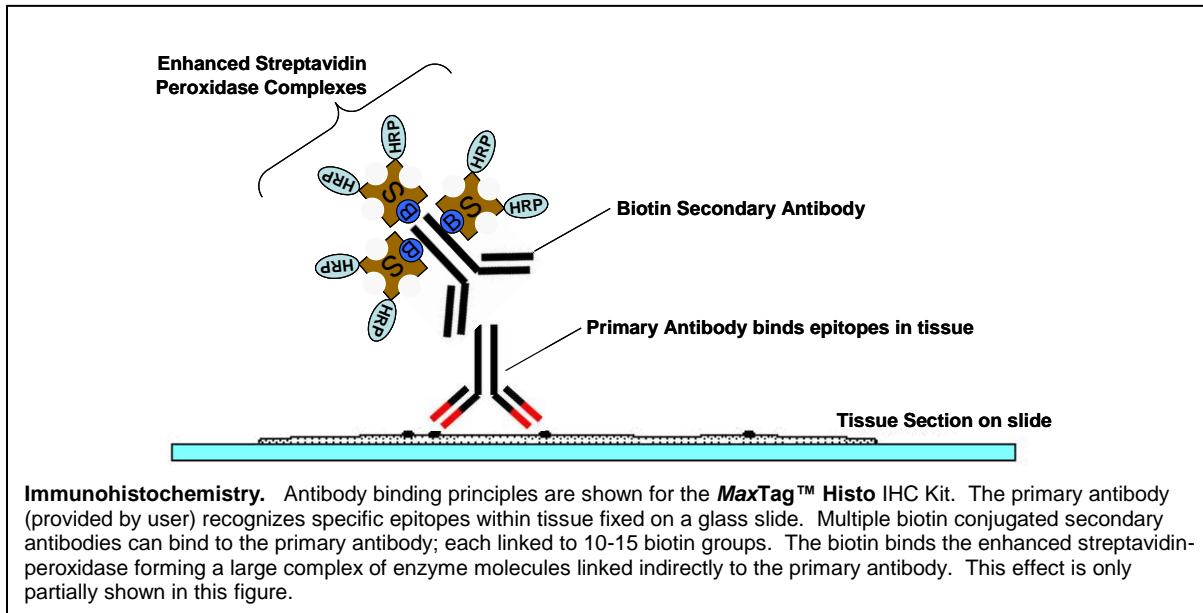
The reagents in **MaxTag™ Histo** Immunohistochemistry Kit are sufficient to run approximately 100-200 immunostains on standard microscope slides. The amount of antibody supplied when diluted as recommended in our protocol will yield 50 mL of working solution.

VI. Kit Components and Layout

1. (5) x 10 mL Formaldehyde (16%) Fixative in snap-open glass ampules
2. (3) x 1 mL Normal Goat Serum in 1 mL dropper bottles with **YELLOW** caps
3. (1) x 0.5 mL Biotinylated anti-Rabbit IgG antibody in 1 mL dropper bottle with **BLUE** cap
4. (1) empty 15 mL dropper bottle with **BLUE** cap labeled "Diluted Secondary antibody"
5. (1) x 0.5 mL Streptavidin peroxidase conjugate in 1 mL dropper bottle with **GREEN** cap
6. (1) empty 15 mL dropper bottle with **GREEN** cap labeled "Diluted Streptavidin Peroxidase"
7. (1) empty 15 mL dropper bottle with **RED** cap labeled "Color Reagent Mixing Bottle"
8. (5) x 2.5 mg DAB Substrate tablets in amber glass vials with **RED** caps
9. (1) x 15 mL Hematoxylin Counterstain in 15 mL dropper bottle with **PURPLE** cap
10. (1) x 10 mL Hydrogen Peroxide 30% solution in a dropper bottle with **PINK** cap
11. (1) x 100 mL bottle 10X Phosphate Buffered Saline in square bottle with **WHITE** cap
12. (1) x 15 mL Polymount Mounting Media in an amber glass bottle with a **BLACK** cap
13. (1) Syringe with needle
14. Instruction Manual.



VII. Immunohistochemistry diagram of IHC reagents binding to tissues



VIII. Materials Required but Not Supplied

Nearly all components required for immunohistochemistry and staining have been provided for your convenience as reagents in the **MaxTag™ Histo** Immunohistochemistry Kit. Some additional materials that may be needed are detailed below:

- ◆ Deionized water
- ◆ Microscope slides
- ◆ Microscope
- ◆ Primary Antibody (Rabbit derived monoclonal or polyclonal)
- ◆ Alternate fixatives
- ◆ Methanol, ethanol, acetone and/or xylene

IX. Preparation of Working Solutions

The **MaxTag™ Histo** Immunohistochemistry Kit comes with concentrated stocks of biotinylated secondary antibody enhanced streptavidin peroxidase conjugate, lyophilized chromogenic substrate, and 10X phosphate buffered saline. Dilute/dissolve these items to working concentrations immediately before use according to the instructions below. For your convenience easy-to-use dropper bottles are provided that have been labeled and color coded to match the respective stock solutions.

- *Preparation of buffers.* The volume of buffer required will depend on the number of slides to be processed. We have included 100 mL of 10X PBS sufficient to produce 1.0 liter of 1X working buffer. Prepare all solutions using deionized water (or equivalent). If desired, pass solutions through a 0.22- μ m filter prior to use. Store diluted solutions at +4°C for a maximum of 3 to 4 weeks. **This buffer does not contain preservative.** Final wash buffers **MUST NOT CONTAIN SODIUM AZIDE** or other inhibitors of peroxidase activity.

Buffer I (PBS Wash Buffer):

Dilute 20 mL of 10X PBS provided in the **WHITE** capped bottle up to 200 mL with deionized water. Mix thoroughly. There is no need to adjust pH. The resultant buffered saline solution is ready-to-use and contains 0.01 M Sodium Phosphate, 0.14 M Sodium Chloride, pH 7.2.

Buffer II (Antibody Dilution Buffer):

This buffer is made up exactly like Buffer I (PBS) with the addition of 1 mL of Normal Goat Serum provided in the **YELLOW** cap dropper per 100 mL final volume.

- *Secondary Antibody.* To prepare 10 mL of the diluted secondary antibody squeeze 2 drops of concentrated biotinylated secondary antibody from the **BLUE** capped dropper bottle into the **BLUE** capped 15 mL bottle labeled "Diluted Secondary Antibody". Add 10 mL of Buffer II and mix thoroughly. This is sufficient for 20 to 40 slides using 0.25 to 0.50 mL of diluted antibody solution per slide. For greater volumes of diluted biotinylated secondary antibody, simply add two drops of concentrate per 10 mL of Buffer II.

- *Enhanced Streptavidin Peroxidase.* To prepare 10 mL of diluted Streptavidin Peroxidase solution squeeze 2 drops of concentrated Streptavidin Peroxidase from the **GREEN** capped dropper bottle into the **GREEN** capped 15-ml bottle labeled "Diluted Streptavidin Peroxidase". Add 10 mL of Buffer I and mix thoroughly. For greater volumes of diluted Streptavidin Peroxidase, simply add 2 drops of concentrate per 10 mL of Buffer I.

- *DAB Substrate.* Reconstitute the contents of one (1) **RED** capped 2.5 mg DAB Substrate vial by adding 5 mL of deionized water directly to the vial resulting in a 0.5 mg/mL solution. Mix thoroughly. Using the provided syringe to minimize "hands-on" contact (see additional notes), transfer the contents of the vial to the **RED** capped empty 15 mL dropper bottle labeled "Color Reagent Mixing Bottle". Temporarily remove and set aside the cap and dropper tip from this bottle. Remove the **PINK** cap from the bottle containing 30% (v/v) Hydrogen peroxide solution and squeeze one drop into the bottom of the **PINK** cap. Using a pipet remove exactly 4 μ L of the hydrogen peroxide from the bottom of the cap and add it to the "Color Reagent Mixing Bottle". Rinse out the **PINK** cap with water and re-cap the bottle containing the hydrogen peroxide. Reinsert the dropper tip and cap into the "Color Reagent Mixing Bottle" and mix thoroughly. Dispense the DAB using this convenient and re-usable container. Discard after 4 to 6 hours. Do not store.

- *Hematoxylin Counterstain and Polymount Mounting Media.* These components are ready-to-use and require no additional preparation.

- *Formaldehyde Fixative.* Carefully snap the neck of the glass ampule containing the formaldehyde fixative. Add 10 mL of fixative to 30 mL of Buffer I and mix thoroughly. The resultant 4% (v/v) solution is ready-to-use.

X. Immunohistochemistry Method

The following method is suggested as a **guideline** for the use of Rockland Immunochemicals' **MaxTag™ Histo** Immunohistochemistry Kit for IHC staining of tissues. This kit provides a very high level of immunological detection yet it is simple and easy to use. Depending on the nature of the user's antigen and primary antibody, specific conditions may be changed as necessary. All reactions can be performed at room temperature or with gentle heating (e.g. 30°C). Use of a rocking platform set at low speed for gentle agitation is suggested. Always add enough solution to cover the sample. A **PAP PEN** may be used to ring the specimen to concentrate the solution onto the sample. Do not let the sample air dry during this process. Do not touch the sample with your skin. **WEAR GLOVES**. Add the reagents to the slides using the provided squeeze bottles.

Sample Preparation:

The best fixation method for a given antigen and antibody has to be determined by trial and error for each antigen under study. Unfortunately, no one universal fixative reagent exists. At best a compromise between preserving cellular structure and maintaining epitope antigenicity can be achieved. Formaldehyde fixation is the routine initial method of choice for tissues and cells and is included as a component of this kit. The following protocol is given for **tissue culture cells fixed with formaldehyde**. See additional notes.

1. Grow cells on glass microscope slides, glass cover slips or slide culture chambers.
2. Remove culture medium and wash cells gently 3 times with ice cold Buffer I.
3. Fix cells by adding a volume of 4% formaldehyde in Buffer I equal to the original volume of culture medium for 30 minutes on ice.
4. Remove the fixative and wash 3 times for 5 minutes each with Buffer I.
5. (Optional) Incubate 5 minutes in 1% H₂O₂ in Buffer I to remove endogenous peroxidase activity.
6. Wash the fixed cells 3 times for 5 minutes each with Buffer I.

Primary Antibody Reaction:

The primary antibody is diluted in serum (GOAT) obtained from the species in which the secondary antibody was raised (GOAT). In this solution the amount of primary antibody relative to total protein is extremely low. Charged proteins present in the serum bind to available sites on cells preventing the antibody reagents from binding to these sites.

1. Prepare the primary antibody by diluting 1:100 in Buffer II. See additional notes.
2. Remove the buffer from the cells. Add a sufficient volume of diluted primary antibody to cover the cells. Incubate with primary antibody for 60 minutes at room temperature. If the primary antibody has a low-affinity for the antigen, then extend the incubation time to overnight at 4°C. Remove primary antibody solution.
3. Wash 3 times for 5 minutes each with Buffer I.

Immunostaining:

1. Remove the buffer from the cells. Using the **BLUE** capped dropper bottle, add "Diluted Secondary Antibody" and incubate for 30 minutes at room temperature. Remove solution.
2. Wash 3 times for 5 minutes each with Buffer I. Remove buffer.
3. Using the **GREEN** capped dropper bottle, add "Diluted Streptavidin Peroxidase" and incubate for 30 minutes at room temperature. Remove solution.
4. Wash 3 times for 5 minutes each with Buffer I. Remove buffer.
5. Using the **RED** capped dropper bottle, add DAB substrate in the "Color Reagent Mixing Bottle" and incubate approximately 10 minutes or until sufficient color develops. Remove solution.

Sample Preservation and Observation:

1. Wash 3 times for 2 minutes each with distilled H₂O.
2. Using the **PURPLE** capped dropper bottle, add hematoxylin counterstain (included) for 1 to 5 minutes depending on the concentration and color intensity desired.
3. Wash 3 times for 2 minutes each with distilled H₂O.
4. Dehydrate the cells with 100% ethanol 4 times for 2 minutes each.
5. Clear the cells with xylene 4 times for 2 minutes each.
6. Add 2 -3 drops of Polymount Mounting Media from the **BLACK** capped amber bottle. Place a cover slip over the sample and allow to air dry.
7. Observe cells under the microscope. A positive reaction should be visible as a brown precipitate. The nuclei should appear light blue.

XI. Additional Notes

- The method given in these instructions is to be used as a guideline. Experienced users can make deviations from the outlined procedure. Note that the solutions have been optimized for the given method and alteration of the reagent concentrations, volumes, reaction times, or temperature will affect the overall performance of the kit. Generally, when modifying conditions experimentally, only alter one variable at a time.
- Store the components of this kit at 4°C.
- Fixation of tissues and cells is highly dependent on the nature of the antigen and antibody to be studied. Generally, adherent tissue culture cells are grown on microscope slides and are fixed with formaldehyde or methanol. Cells in suspension cultures may be centrifuged on to a slide using a cytospin centrifuge and then fixed with ethanol. Frozen tissue sections are generally mounted on slides and fixed with acetone. Tissues not sufficiently rigid to withstand sectioning may be embedded in paraffin wax or resin and sectioned prior to mounting on slides and then de-paraffinized with xylene followed by ethanol. Fixatives generally either coagulate proteins (solvents such as alcohols and acetone) or crosslink proteins (formaldehyde, glutaraldehyde and resins). Fixatives that minimally affect antigenic properties of tissues and cells generally do not preserve morphology well. Conversely, methods that preserve detailed morphology usually destroy or diminish the reactivity of antigens with antibodies. The loss of antigenicity increases with fixative concentration and time of fixation. Pre-treating tissues with non-ionic detergents or enzymes such as pronase or trypsin may enhance permeability of tissues to immunochemical reagents. Refer to the given references for more details.
- The dilution of primary antibody with Buffer II is estimated at 1:100. The optimal dilution should be determined by trial and error. For beginning titrations of antibodies with unknown properties, start at 1:10 and do serial dilutions resulting in 10-, 100-, 1,000- and 10,000 - fold dilutions of concentrated antibody.
- Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used to dilute the streptavidin peroxidase conjugate or in washes subsequent to the addition of the peroxidase conjugate.
- The chromogenic substrate DAB is a potential carcinogen and should be handled accordingly. Use the provided syringe to minimize "hands on" contact. DAB solutions and spills can be neutralized with 30% bleach.
- The individual components of this kit may be ordered separately.

XII. Troubleshooting Guide

Troubleshooting:

Problem: No (weak) staining.

Cause: Poor binding of primary antibody. Primary antibody may be present in too low concentration. Decrease the dilution of your primary antibody.

Target antigen may be present in too low concentration to detect. Try again with another section.

Primary antibody may be inactivated or inappropriate for the antigen. Contact the manufacturer of your primary antibody.

Poor binding of biotinylated secondary antibody. Be sure the source of the primary antibody is matched with the target of the biotinylated secondary antibody included in this kit.

Streptavidin-peroxidase conjugate is inactivated. Be certain that all the buffers are free of sodium azide. Azide is a strong inhibitor of peroxidase activity.

Problem: Staining is too heavy.

Cause: Concentration of primary antibody is too high. Increase dilution factor.

Over development of the substrate in the detection step. In most cases, full color development is achieved in 5-10 minutes. The reaction should be stopped after 20 minutes to prevent false positives.

Problem: High Background/Poor Signal-to-Noise Ratio.

Cause: Non-specific binding of the primary or secondary antibody. Be sure that the dilution buffer has been properly prepared. Antibodies should be diluted in a solution that contains serum proteins from the same species as the antigen being detected. These should be present in concentrations of 1% to 5%.

Insufficient washing.

XII. References

Antibodies, A Laboratory Manual. Ed Harlow and David Lane, eds. Cold Spring Harbor Press, 1988. Chapter 10 discusses various immunohistochemical techniques.

Immunocytochemical Methods and Protocols. L.C. Javois, ed. Methods in Molecular Biology series Volume 34. Humana Press, 1994. Refer to Parts II and III.

Antibody Techniques. V.S. Malik and E.P. Lillehoj, eds. Academic Press, 1994. Chapter 11 discusses Immunostaining cells and tissues.

Immunochemical Protocols. M.M. Manson, ed. Methods in Molecular Biology series Volume 10. Humana Press, 1992. Refer to Chapter 11.

XIV. Trademarks

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XV. Disclaimer

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XVI. Additional Products and Services

Additional Immunohistochemistry **MaxTag™** Histo IHC Kits

Product	Code	Size	Price
MaxTag™ Histo Immunohistochemistry Kits for IHC using Human Primary Antibody	KHA001	1 each	inquire
MaxTag™ Histo Immunohistochemistry Kits for IHC using Mouse Primary Antibody	KHA002	1 each	inquire
MaxTag™ Histo Immunohistochemistry Kits for IHC using Rabbit Primary Antibody	KHA003	1 each	inquire
MaxTag™ Histo Immunohistochemistry Kits for IHC using Goat Primary Antibody	KHA004	1 each	inquire

Replacement Parts for Immunohistochemistry **MaxTag™** Histo IHC Kits

Product	Code	Size	Price	Product	Code	Size	Price
Biotinylated Anti-Human IgG	KHB001	1 mL	inquire	DAB Substrate Tablets	DAB-50	50 tabs	inquire
Biotinylated Anti-Mouse IgG	KHB002	1 mL	inquire	Normal Goat Serum	D304	5 ml	inquire
Biotinylated Anti-Rabbit IgG	KHB003	1 mL	inquire	Formaldehyde Fixative	KHF001	5 x 10 ml	inquire
Biotinylated Anti-Goat IgG	KHB004	1 mL	inquire	Hematoxylin Counterstain	KHG001	15 ml	inquire
<i>Enhanced</i> Streptavidin HRP	KHD001	1 mL	inquire	Polymount Mounting Media	KHH001	15 ml	inquire

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