

MaxTag™ Histo Kit
for use with Human Primary Antibody

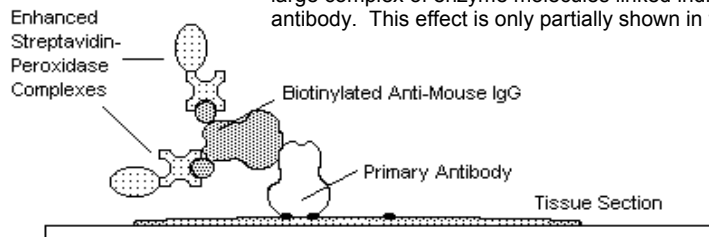
Product Overview

The **MaxTag™ Histo** kit is intended to provide a simple, reliable and convenient detection system for immunohistochemical staining to 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 that can be reacted with a suitable substrate to give a colored product. Some advantages of immunohistochemical staining are that nuclei can be counterstained, thereby revealing the tissue architecture, and that the stain fades 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 protocol is to be used as a guideline: remember that any procedure can be altered according to specific experimental requirements. The use of highly purified and adsorbed antibody conjugated and matched reagents reduces any lot-to-lot variation. **Please read the entire product insert before you begin.**

Intended Use

Use the **MaxTag Histo** kit for the detection of primary antibodies complexed with antigens immobilized on glass microscope slides and the visualization of these antibodies using a chromogenic substrate.

Figure 1. The primary antibody recognizes specific epitopes on tissue fixed on a glass slide. Several biotinylated secondary antibody molecules can bind to the primary antibody, each linked to 10-15 biotin groups. The biotin binds the enhanced streptavidin-peroxidase forming a large complex of enzyme molecules linked indirectly to the primary antibody. This effect is only partially shown in this figure.



Kit Principle

This kit relies upon the high specificity of avidin-biotin binding for visualization of an antigen. The antigen (usually 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 primary antibody raised in Human reacts with the complex. Streptavidin conjugated to Horseradish Peroxidase reacts with this complex immobilizing the peroxidase at the site of the antigen. Finally, substrate is added causing a colored precipitate to form on the slide at the location of the antigen. This may now be viewed through a light microscope.

Number of Assays

Components in this 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.

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.

Kit Components:

1. Five (5) x 10 ml Fixative, 16% (w/v) formaldehyde in glass ampules
2. Normal Goat Serum, 3x1 ml, in 1ml dropper bottles with a white cap.
3. Biotinylated secondary antibody (anti-Human IgG in Goat), 0.5 ml, in a 1 ml dropper bottle (blue cap).
4. Streptavidin peroxidase conjugate, 0.5 ml in a 4 ml dropper bottle (red cap).
5. One (1) empty 15 ml dropper bottle (blue cap), labeled "Diluted Secondary antibody".
6. One (1) empty 15 ml dropper bottle (red cap), labeled "Diluted Streptavidin Peroxidase".
7. One (1) empty 15 ml dropper bottle (orange cap), labeled "Color Reagent Mixing Bottle".
8. Hematoxylin Counterstain in 15 ml dropper bottle (white cap).
9. Five (5) vials containing 2.5 mg lyophilized DAB Substrate (red cap, amber vial)
10. One (1) 10 ml bottle (white cap) 30% (v/v) Hydrogen Peroxide solution.
11. One (1) 100 ml bottle 10X Phosphate Buffered Saline.
12. One (1) 15 ml bottle Polymount Mounting Media.
13. One (1) 5 ml Syringe with needle.
14. Instruction Manual.

Materials Required but not Supplied:

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

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

Preparation of Working Solutions:

The **MaxTag Histo** kit comes with concentrated stocks of biotinylated secondary antibody, enhanced streptavidin peroxidase conjugate, lyophilized chromogenic substrate and packets containing buffer salts. 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 buffers required will depend on the number of slides to be processed. We have included foil packets containing buffered phosphate and salts to facilitate buffer preparation. Prepare all solutions using deionized water (or equivalent). If desired, pass solutions through a 0.22 μm filter prior to use. Store diluted solutions at +4°C for a maximum of 3-4 weeks. 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 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.

Buffer II (Antibody Dilution Buffer):

This buffer is made up exactly like Buffer I (PBS) with the addition of 1 ml Normal Goat Serum (provided) 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 red capped dropper bottle into the red 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 Buffer I.

- *DAB Substrate.* Reconstitute the contents of one (1) blue 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. Use the provided syringe to minimize “hands-on” contact. See additional notes. Mix thoroughly. Transfer the contents of the vial to the blue capped empty 15 ml dropper bottle labeled “Color Reagent Mixing Bottle”. Add 4 μ l of 30% (v/v) Hydrogen peroxide solution. 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.

Procedure:

The **MaxTag Histo** Kit provides a very high level of immunological detection yet it is simple and easy to use. The following method is a guideline. 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. Do not let the sample air dry during this process. Do not touch the sample with your skin. **WEAR GLOVES.** Reagents may be added to the slides conveniently with 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 coverslips or slide culture chambers
2. Remove culture medium and wash cells gentle 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 obtained from the species in which the secondary antibody was raised. In this solution the amount of primary antibody relative to total protein is extremely low. Charged proteins present in the serum to 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, incubate at 4° C overnight. Remove primary antibody solution.
3. Wash 3 times for 5 minutes each with Buffer I.

Immunostaining:

1. Remove the buffer from the cells. 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. 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. Add DAB substrate using 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. Counterstain with hematoxylin (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, add coverslip 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.

Additional Notes:

- The method given in these instructions is to be used as a guideline. Deviations from the outlined procedure can be made by experienced users. 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. Permeability of tissues to immunochemical reagents may be enhanced by pre-treating tissues with non-ionic detergents or enzymes such as pronase or trypsin. 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.
- Store the components of this kit at 4°C.
- The individual components of this kit may be ordered separately.

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.

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 which contains serum proteins from the same species as the antigen being detected. These should be present in concentrations of 1% to 5%.

Insufficient washing.

MaxTag™ Histo Replacement Parts List

Product	Code	Size	Price	Product	Code	Size	Price
Biotinylated Anti-Human IgG	KHB001	1 ml	\$70	<i>Enhanced</i> Streptavidin HRP	KHD001	1 ml	\$70
Biotinylated Anti-Mouse IgG	KHB002	1 ml	\$70	Normal Goat Serum	D304	5 ml	\$12
Biotinylated Anti-Rabbit IgG	KHB003	1 ml	\$70	PBS packets (1000 ml)	MB-0032	1 pouch	\$10
Formaldehyde Fixative	KHF001	5 x 10 ml	\$20	DAB Substrate Powder	DAB-525	5 x 2.5 mg	\$45
Hematoxylin Counterstain	KHG001	15 ml	\$20	Polymount Mounting Media	KHH001	15 ml	\$20