ROCKLAND
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*Max*Tag™ Kit for Immunoblotting

For use with Mouse Primary Antibody

Overview

Rockland's ***Max*Tag**™ immunoblotting kit provides a rapid, sensitive, and economical way to develop your immunoblots. The design of this kit simplifies the process of immunoblotting applications. Straightforward procedures and color-coding add to the ease of use. Two substrates are provided for flexibility, TMB substrate producing a bright blue color and DAB substrate producing a dark brown color. This kit can process up to 40 blots (100 cm^2) and is stable for at least 1 year when stored at +4°C. **Please read the entire product insert prior to use.**

Kit Principle

This kit allows for the detection of primary Mouse polyclonal or monoclonal antibody provided by the user. After protein separation and transfer, the membrane is probed with primary antibody. The first step in the detection of the membrane bound primary antibody-antigen complex is the addition of a secondary antibody that has been affinity purified, cross-adsorbed, and biotinylated. This biotinylated secondary antibody is visualized after reaction with streptavidin-peroxidase conjugate and subsequent addition of either TMB or DAB substrates.

The increased sensitivity and low background achieved by Rockland's ***Max*Tag**™ Immunoblotting Kit relies on the use of an enhanced streptavidin-peroxidase conjugate and a highly active biotinylated antibody. The streptavidin carries a neutral charge and fewer carbohydrate groups than positively charged avidin. The biotin incorporates a "spacer" group that allows for better accessibility of the streptavidin-peroxidase conjugate.

Intended Use

Use Rockland's ***Max*Tag**™ Immunoblotting Kit for the detection of Mouse polyclonal or monoclonal antibody complexed with antigen immobilized on a membrane (immunoblot) using the substrate TMB or DAB. This Kit is useful for both “western blotting” and “dot blotting” methods. Nitrocellulose, nylon or other membrane formats are all suitable for use (see “Additional Notes”). Please call your technical representative for further assistance.

Number of Assays

Components in this kit are sufficient to run approximately 40 immunoblots (10 cm x 10 cm). The amount of antibody supplied when diluted as recommended in our protocol will yield 200 ml of working solution. Adjustments in volumes for larger or smaller blots will effect the number of blots detected.

USDA Certification

All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

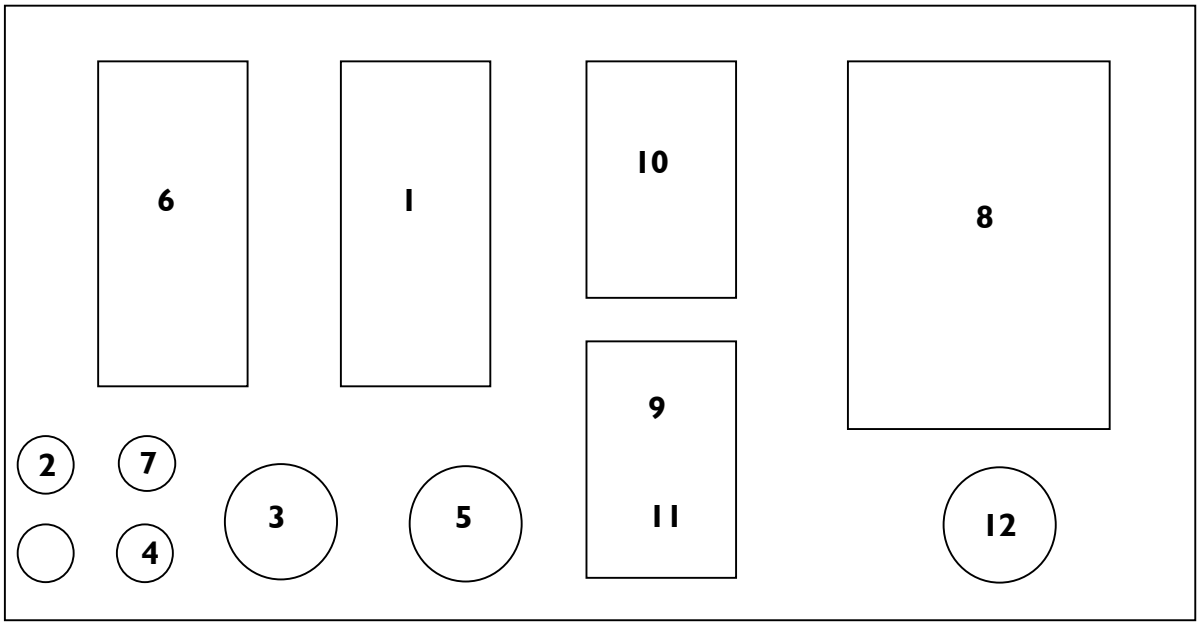
Storage and Stability

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.

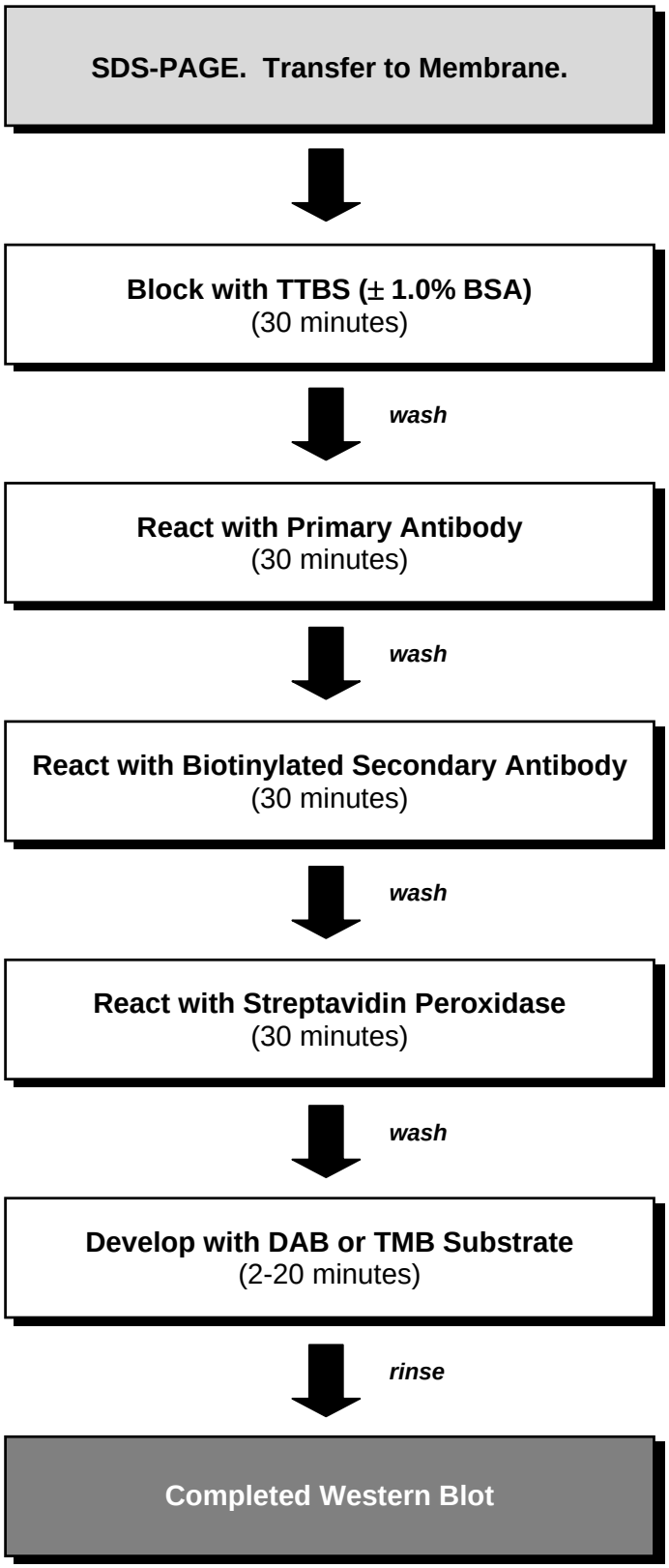
Kit Components

1. (1) x 50 ml Ultra Pure Tween-20 in an amber glass bottle with **BLACK** cap
2. (1) x 1.0 ml Biotinylated Goat anti-Mouse IgG antibody in 1 ml dropper bottle with **BLUE** cap
3. (1) empty dropper bottle with **BLUE** cap labeled “Diluted Secondary antibody”
4. (1) x 1.0 ml Streptavidin peroxidase conjugate in 1 ml dropper bottle with **RED** cap
5. (1) empty dropper bottle with **RED** cap labeled “Diluted Streptavidin Peroxidase”
6. (1) x 10 g Bovine Serum Albumin (BSA) in a plastic bottle with a **WHITE** cap
7. (1) x 0.5 ml Control Mouse IgG in plastic vial with **YELLOW** cap
8. (1) x 100 ml TMB Substrate in an amber glass bottle with **BLACK** cap
9. (1) x 10 ml TMB Substrate Precipitating Agent in an amber glass bottle with **BLACK** cap
10. (1) x 50 tablets DAB substrate (tabs) in an amber glass bottle with **BLACK** cap
11. (1) x 10 ml Hydrogen Peroxide 30% solution in a dropper bottle with **PINK** cap
12. (1) empty dropper bottle with **GREEN** cap labeled “Substrate Mixing Bottle”
13. Instruction Manual

Component Layout



Flow Diagram of *Max*Tag™ Kit Procedure



Materials Required but Not Supplied

Nearly all components required for immunoblotting are provided for your convenience in Rockland's ***Max*Tag**™ Immunoblotting Kit. Some addition materials are required:

- SDS-PAGE electrophoresis materials
- Nitrocellulose or other membrane for protein transfer and transfer materials
- Primary Antibody (Mouse derived monoclonal or polyclonal)
- Rocker platform gentle mixing during incubations
- Incubation trays
- Deionized water

Solutions Required but Not Supplied

The user is to prepare the following buffers for this procedure. The exact volume of buffers required depends on the size of the membranes to be processed. We suggest preparation of 1.0 L of Tris Buffered Saline with Tween (TTBS) and 100 ml of TTBS with BSA. Prepare all solutions using ultra pure reagents and deionized (or equivalent) water. Filter the solutions and store at +4° C. Warm solutions to room temperature prior to use. Do not store solutions for more than one (1) month. Final wash buffers *MUST NOT CONTAIN SODIUM AZIDE* or other inhibitors of peroxidase activity.

Buffer I **Tris Buffered Saline with Tween-20 (TTBS)**

Add 800 ml of deionized water
Dissolve 12.1 g of Tris base
Dissolve 8.8 g Sodium Chloride (NaCl)
Adjust pH to 7.5 with HCl
Add 1.0 ml of Tween-20 (provided)
Adjust volume to 1.0 L with deionized water

Buffer II **TTBS with 1.0% (w/v) BSA**

Add 100 ml of TTBS
Dissolve 1.0 g of BSA (provided)
Use immediately

Preparation of Working Solutions

The ***Max*Tag**™ kit comes with concentrated stocks of biotinylated goat anti-Mouse antibody and streptavidin peroxidase conjugate. Prior to use these solutions must be diluted to working solutions. Substrates must be prepared as working solutions immediately prior to use. Dropper bottles are provided for ease-of-use and are labeled and color coded to match the respective dilution bottles. Just add the appropriate volume of concentrated stock solution (as described below) to 10 ml of buffer. Replace the dropper tip insert and cap, mix the solution, and the working solution is ready for use.

- **Secondary antibody.** To prepare 10 ml of diluted secondary antibody solution add 1 drop (~50 µl) of concentrated Biotinylated goat anti-Mouse antibody from the **BLUE** capped 1.0 ml dropper bottle to the **BLUE** capped dropper bottle labeled “Diluted Secondary Antibody." Add 10 ml of Buffer I (TTBS). Mix thoroughly. For greater volumes simply add 1 drop of concentrate per 10 ml of *Buffer I*.
- **Streptavidin Peroxidase.** To prepare 10 ml of diluted Streptavidin-Peroxidase solution add 1 drop (~50 µl) of concentrated enhanced Streptavidin-Peroxidase from the **RED** capped 1.0 ml dropper bottle to the **RED** capped dropper bottle labeled “Diluted Streptavidin-Peroxidase." Add 10 ml of Buffer I (TTBS). Mix thoroughly. For greater volumes simply add 1 drop of concentrate per 10 ml of *Buffer I*.

- **Substrate Solution.** Decide on whether you prefer to use the TMB substrate for bright blue color or the DAB substrate for dark brown color. Only prepare one substrate per experiment. Follow the appropriate instructions below for each substrate. See the Notes section for precautions concerning the use of DAB.

  For TMB Substrate Solution. Locate the TMB Substrate in the **BLACK** capped 100-ml bottle, the TMB Precipitating Agent in the **BLACK** capped 10-ml bottle, and the **GREEN** capped dropper bottle labeled "Substrate Mixing Bottle." Remove both the cap and the tip insert from the dropper bottle and add 9 ml of the TMB Substrate and 1.0 ml of the TMB Precipitating Agent. Replace the tip insert and the cap and mix thoroughly. The substrate is now ready to use. Be certain to use within 24 hours.

  For DAB Substrate Solution. Locate the DAB tablets in the **BLACK** capped amber glass bottle, the Hydrogen Peroxide (H_2O_2) solution in **PINK** capped dropper bottle, and the **GREEN** capped dropper bottle labeled "Substrate Mixing Bottle." Remove both the cap and the tip insert from the Substrate Mixing Bottle and add one (1) DAB tablet and 10 ml of deionized water. Allow approximately 5 minutes for the tablet to dissolve completely. Add one (1) drop of the Hydrogen Peroxide solution. Replace the tip insert and the cap and mix thoroughly. The substrate is now ready to use. Discard after 6 hours.

Immunoblot Method

The following method is suggested as a **guideline** for the use of Rockland's ***Max*Tag**™ Immunoblotting Kit with nitrocellulose or PVDF membranes. Nylon membranes may also be used (see "Additional Notes"). Some primary antibodies and/or antigens may require specific conditions other than those stated below. After your antigen has been immobilized onto the membrane by transfer, dotting or filtration, follow the numbered steps below to process the immunoblot. All reactions occur at room temperature. Use a rocking platform set at low speed for gentle agitation. Always add enough solution to cover the membrane. Never let a membrane air dry during this process. Add the suggested volumes or just enough volume to cover the membrane to keep it wet. Do not touch the membrane with your skin! Wear gloves.

1. Immerse the membrane in *Buffer II* (TTBS with BSA). Incubate for 30 min with gentle agitation. The addition of 1.0% BSA (provided) in this first blocking step increases the signal-to-noise ratio when compared to using TTBS alone.
2. Aspirate the blocking solution[1]. Immediately add 5 ml of primary antibody solution (not provided) diluted in *Buffer I* (TTBS). The appropriate dilution should be determined by the end user. If unknown, a starting dilution of 1:500 to 1:1,000 is suggested. Greater dilutions often result in lower backgrounds but may require longer incubation times. Incubate for 30 min.
3. Aspirate the primary antibody solution. Wash the blot with 3 changes of *Buffer I* for 5 min each.
4. Aspirate the wash solution and add 5 ml of the "Diluted Secondary Antibody" solution from the **BLUE** capped dropper bottle (see above for preparation). Incubate for 30 min.
5. Aspirate secondary antibody solution. Wash the blot as in Step 3.
6. Aspirate the wash solution and add approximately 5 ml of the "Diluted Streptavidin-Peroxidase" solution from the **RED** capped dropper bottle (see above for preparation). Incubate for 30 min.
7. Aspirate Streptavidin-Peroxidase solution. Wash the blot as in Step 3.
8. Aspirate the wash solution and add approximately 5 ml of freshly prepared substrate solution, either TMB or DAB, from the **GREEN** capped dropper bottle (see above for preparation). Incubate until color develops (usually 2 to 20 min).
9. Wash the membrane with 2 changes of deionized water for 5 min each. Allow the membrane to dry and store for future analysis in the dark.

[1] Aspirate using a glass pipette attached to a vacuum. Alternatively, the solution may be poured off away from the immunoblot.

Additional Notes

- The methods given in these instructions are to be used as a guideline. Experienced users can make deviations from the stated method. Solutions have been optimized for the stated method and any change in reagent concentration, volume, or reaction time or temperature will effect the overall performance of the kit. Generally, if a variable is to be modified, only alter one condition at a time.
- Nylon membrane is more difficult to block and may result in higher levels of non-specific staining. Using 10% non-fat dry milk is suggested to block nylon membranes. Allow the blocking step to proceed for several hours to overnight at 37° C. Do not use Tween-20 when using nylon membranes.
- The blocking of membranes for immunoblotting can be accomplished with TTBS only. We suggest adding BSA to lower non-specific staining. Users may omit BSA from the blocking step or use some other blocking agent, such as normal goat serum, fish gelatin, or other commercially available blocking agent, depending on previous experience.
- Some primary antibodies do not bind well in the presence of mild detergents like Tween-20. In these instances, replace the TTBS with TBS or PBS containing BSA or 1% to 10% normal goat serum (secondary antibody is goat host).
- Allows use enough solution to cover the membrane. Never let the membrane dry during the process.
- Use care not to touch the membrane with your skin! Wear gloves.
- All reactions occur at room temperature.
- Use a rocking platform set at low speed for gentle agitation for all incubation steps.
- DAB is a suspected carcinogen. Always wear gloves, eye protection, and lab coats. Use good laboratory procedures when handling DAB tablets and solutions. Dispose of DAB in accordance with local regulations.
- Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used to dilute the streptavidin peroxidase, substrates or any other ***Max*Tag**™ reagent.
- A control Mouse IgG is provided in a YELLOW capped vial for use to ensure the ***Max*Tag**™ kit components are performing as described. Spot 1 or 2 μl as a control on your western or dot blot prior to the blocking step.
- Store the components of this kit at +4° C.
- Individual components of this kit may be ordered separately.

Troubleshooting Guide

Little or no signal

Incomplete transfer of proteins. Follow all protocols included with your transfer apparatus. Check for the presence of transferred proteins using India Ink stain as described in Reference 1.

Poor binding of primary antibody. Decrease the dilution (increase the concentration) of your primary antibody. Increase the incubation time of the primary antibody solution from 30 minutes to several hours or overnight. Increase the incubation temperature to 37° C. If all of the above fails, contact the source of your primary antibody.

Poor binding of biotinylated anti-IgG. Be sure the source of the primary antibody is appropriate for this kit. Include 1 or 2 µl of Mouse IgG in a YELLOW capped vial as a control in your western blot or dot blot to ensure that the ***Max*Tag**™ Kit components are performing as described.

Inactive Streptavidin Peroxidase. Be certain that all buffers are free of sodium azide, which is a strong inhibitor of peroxidase activity.

Multiple signals

Too much protein on the blot. Verify the concentration of your protein sample, using Bradford or BCA reagent. For best results, load approximately 10 µg of total protein per lane.

Too high concentration of primary antibody. Increase the dilution of primary antibody solution.

Too much time in substrate solution. Stop color reaction as soon as the desired bands have been visualized. All substrates should be stopped after 20 min to prevent "false positives."

High background / Poor signal-to-noise ratio

Insufficient blocking. Be certain blocking buffer has been properly prepared. In most cases, the addition of 1.0% BSA will decrease background over the use of TTBS alone. In some cases, increased concentrations of BSA (up to 5%) are necessary.

Insufficient Washing. Increase the number of wash steps and the volume of TTBS used for each wash.

References

Antibodies, A Laboratory Manuel. Ed Harlow and David Lane, eds. Cold Spring Harbor Press. 1988. Chapter 12 gives an excellent overview of Western Blotting techniques, including India Ink staining.

Current protocols in Molecular Biology. J. Ausebel, et al, eds. John Wiley and Sons, New York. Gives a complete protocol of Western Blotting and Dot Blotting.

Molecular Cloning: A Laboratory Manuel. 2nd Edition. J. Sambrook, E.F. Fritsch and T. Maniatis, eds. Cold Spring Harbor Press, 1989. Chapter 18 gives detailed protocols for both the production of cell lysates and electrophoresis and blotting of proteins.

Antibodies, A Practical Approach. 2nd Edition. Catty, D., ed. IRL Press, Oxford, England. 1990. Volumes I and II represent a detailed and complete reference for most current antibody techniques.

Additional Products

Product	Code	Size	Price
***Max*Tag™** Anti-Human IgG Kit for Immunoblotting	KIA001	1 each	$205
***Max*Tag™** Anti-Mouse IgG Kit for Immunoblotting	KIA002	1 each	$205
***Max*Tag™** Anti-Rabbit IgG Kit for Immunoblotting	KIA003	1 each	$205
***Max*Tag™** Anti-Goat IgG Kit for Immunoblotting	KIA004	1 each	$205
***Max*TagHisto™** Anti-Mouse IgG Kit for Immunohistochemistry	KHA002	1 each	$205

***Max*Tag^{tm} Replacement Parts List**

Product	Code	Size	Price	Product	Code	Size	Price
Biotinylated Anti-Human IgG	KIB001	1 ml	$80	Control Mouse IgG	KIC002	0.5 ml	$25
Biotinylated Anti-Mouse IgG	KIB002	1 ml	$80	BSA, Protease and IgG Free	BSA10	10 g	$35
Biotinylated Anti-Rabbit IgG	KIB003	1 ml	$80	Ultra Pure Tween-20	TW0020	50 ml	$20
Biotinylated Anti-Goat IgG	KIB004	1 ml	$80	DAB Substrate Tablets	DAB-50	50 tabs	$50
Enhanced Streptavidin HRP	KID001	1 ml	$80	TMB Substrate (2 components)	TMBM-101	110 ml	$80

User Notes