INTRODUCTION

Zymed’s HISTOSTAIN®-ER/PR KIT is designed to reveal the presence of Estrogen Receptor (ER) and Progesterone Receptor (PR) antigens in human tissue or cell preparations. Each stain requires a separate sample - this is not a double staining kit. These kits incorporate HorseRadish Peroxidase (HRP), streptavidin, and affinity-purified antibodies into the Labeled-Streptavadin-Biotin (LAB-SA) method, also known as Streptavidin-Biotin Amplification. After the primary antibody has been incubated with a sample, the LAB-SA methodology is now widely used in basic research and routine histology labs.

GOOD FOR: 30 ER + 30 PR slides
CHROMOGEN DAB
CAT. NO. 95-6560

IMMUNOHISTOCHEMISTRY REFERENCES


TRADEMARKS

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HANDLING STORAGE AND SHELF LIFE

Store kit at 2-8°C. All performance claims are void after kit expiration date. Observe necessary health and safety precautions when using the kit. Avoid contact with skin and clothes. Since there is a potential hazard of explosion due to the reaction of sodium azide with copper and metal in the plumbing system, flush the drain thoroughly with water after disposal of reagents.
INTENDED USE
The HISTOSTAIN-ER/PR kit is designed to reveal the Estrogen Receptor and Progesterone Receptor antigens on human tissue or cell samples. This kit is not intended for clinical or diagnostic use.

DESCRIPTION
The HISTOSTAIN-ER/PR kit uses a biotinylated second antibody, a horseradish peroxidase-streptavidin conjugate, and a substrate-chromogen mixture to demonstrate antigen in cells or tissues. The principle of this staining system is as follows:
1) Paraffin embedded tissue should be deparaffinized and rehydrated. Endogenous peroxidase activity can be quenched by treating tissue sections with 3% hydrogen peroxide in absolute methanol or with Peroxo-Block™ (Zymed Cat. No. 00-2015).
2) Non-specific background is eliminated by blockers that are added to the primary antibodies.
3) The ER or PR antibody (Reagent 1A or 1B) will detect a specific antigen in the tissue. Use one antibody per sample.
4) A biotinylated secondary antibody (Reagent 2), which serves as a linker between the primary antibody and the streptavidin-peroxidase conjugate.
5) Streptavidin-peroxidase (Reagent 3), binds to the biotin residue on the linking antibody.
6) The presence of peroxidase is revealed by addition of substrate-chromogen solution (Reagent 4A,B,C; see procedure at right). Peroxidase will catalyze the substrate (hydrogen peroxide) and convert the chromogen (DAB) to a brown deposit, which demonstrates the location of the antigen.

REAGENTS SUPPLIED
Reagent 1A. One dropper bottle (3 ml) of ready-to-use Mouse anti-ER antibody. Do not dilute.
Reagent 1B. One dropper bottle (3 ml) of ready-to-use Mouse anti-PR antibody. Do not dilute.
Reagent 2. One dropper bottle (8 ml) of ready-to-use biotinylated secondary antibody.
Reagent 3. One dropper bottle (8 ml) of ready-to-use streptavidin-peroxidase conjugate.
Reagent 4A. One dropper bottle (1 ml) of concentrated substrate buffer (20x).
Reagent 4B. One dropper bottle (1 ml) of concentrated chromogen solution (20x), (DAB).
Reagent 4C. One dropper bottle (1 ml) of 0.6% hydrogen peroxide (20x).
Reagent 4D. One dropper bottle (8 ml) of ready-to-use Hematoxylin solution.
Reagent 5. One bottle (10 ml) of ready-to-use Histomount. Do not dilute.

REAGENTS AND MATERIALS REQUIRED
But not provided:
- Xylene, ethanol, and absolute methanol
- Distilled or deionized water
- 30% hydrogen peroxide
- 10 mM phosphate-buffered saline, pH 7.4 (PBS)
- 0.01 M citrate buffer (Zymed Cat. No. 00-5000)

SUGGESTED STAINING PROTOCOL
A. PRELIMINARY PREPARATION OF SLIDES.
1. SPECIMEN PREPARATION: Appropriate tissue and antigen fixation is required to obtain reproducible performance and reliable interpretations. Fixation methods for the antigen of interest may be available from current literature. The following are commonly used fixatives:
   - 10% neutral buffered formalin, B5, Bouin’s, Zinc formalin or alcohol-base fixatives are regarded as suitable fixatives for most antigens of clinical significance.
   - Formalin-fixed tissues post-fixed in B5 before paraffin embedding may show improved stain. Cell smears prepared from body fluids should be made to assure a monolayer of cells. Multilayers of cells can trap staining reagents and interfere with the interpretation of the results. Smears should be fixed immediately after preparation.
   - Depending on the properties of the antigen, cell smears are usually stable for two to four weeks when stored at 4°C.
   - Fixation of cytospin or frozen sections can be done with acetone (100%) at 4°C for a period of 10 minutes.
2. SLIDE PREPARATION: Precoat slides with Histogrip™ (Zymed Cat. No. 00-8050). As an alternative, precoat with 0.1% poly-L-lysine in water, then air dry.
3. DEPARAFFINIZATION AND REHYDRATION: Paraffin sections are deparaffinized with xylene and rehydrated in a graded series of ethanol. Wash cell smears or tissue in a PBS bath for 10 minutes before starting the staining procedure.
   Note: Do not allow specimen or tissue sections to dry from this point on.
4. CONTROL SLIDES: Three control slides are necessary for the interpretation of results:
   - Positive Tissue Control: A specimen, processed in the same way as the unknown, contains the antigen to be stained.
   - Reagent Control/Negative Control-1: An additional slide that will be treated with a non-immune serum instead of the primary antibody. Any staining observed on the specimen is probably due to non-specific protein binding or non-specific binding of other reagents.
   - Negative Control-2: A specimen, processed in the same way as the unknown, does not contain the antigen to be stained [optional].

B. REAGENT PREPARATION

C. STAINING PROCEDURES (Do all steps at room temperature)

PEROXIDASE QUENCHING SOLUTION: (Not provided)
This step is optional. Do step 1 if elimination of endogenous peroxidase activity is necessary (i.e. bloody tissue).

For paraffin-embedded tissues, add 1 part of 30% hydrogen peroxide to 9 parts of absolute methanol. Mix well. Not recommended for frozen tissues.

ANTIGEN RECOVERY:
1. (Optional) Submerge slides in PEROXIDASE QUENCHING SOLUTION. Wash with PBS (2 min., 3 times) or For frozen tissue sections, treat with Peroxo-Block (Zymed Cat. No. 00-2015) for 45 sec. Wash immediately with PBS (2 min., 3 times)

2. Immerse slides in 0.01 M citrate buffer and heat at 95-98° C for 10 minutes. Let cool for 10-30 minutes. Rinse slides in PBS.
3. Apply 2 drops or 100 µl PRIMARY ANTIBODY to each section. Incubate. Rinse well with PBS. (2 min., 3 times) One antibody per sample.

4. Apply 2 drops or 100 µl of BIOTINYLATED SECOND ANTIBODY to each section. Incubate. Rinse well with PBS. (2 min., 3 times) One antibody per sample.

5. Apply 2 drops or 100 µl of ENZYME CONJUGATE to each section. Incubate. Rinse well with PBS. (2 min., 3 times)

6. Apply 2 drops or 100 µl of SUBSTRATE-CHROMOGEN MIXTURE to each section. Incubate. Rinse well with distilled water.

7. Counterstain the slides with 2 drops or 100 µl of HEMATOXYLIN. Wash slides in tap water. Put slides into PBS until blue (approx. 30 seconds). Rinse in distilled water.

8. Dehydrate slides with graded series of alcohol, clear in xylene. Add 2 drops or 100 µl of Histomount to the slide and mount with coverslip.

INCUATION TIME (Min.)

10 (Optional)