ABC Peroxidase Staining Kit

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Thermo Scientific™ ABC Peroxidase Staining Kit uses the avidin-biotin complex (ABC) method to amplify signal intensity. The multiple binding sites between the tetravalent avidin and biotinylated antibodies (bound to the antigen) are ideal for improving sensitivity by increasing the number of enzyme molecules at the target. This method uses a biotinylated horseradish peroxidase (HRP) that is pre-incubated with avidin at a specific ratio, forming large complexes. An aliquot of this solution is then added to the biotinylated antibody that is already bound to the target. The result is a greater concentration of enzyme (three enzyme molecules to one avidin molecule) at the antigenic site and, therefore, an increase in signal intensity and sensitivity. This signal amplification method may be used for immunohistochemistry, ELISA and Western blotting.

Contents and storage

Contents	Amount	Storage
Reagent A, Specially-purified Avidin	2mL	Upon receipt store all reagents at 4°C. Product is shipped at ambient temperature.
Reagent B, Specially-prepared Biotinylated Peroxidase	2mL	

Procedural guidelines

- Use ultrapure water in all solutions to prevent microbial growth.
- Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
- Carefully handle the kit reagents to prevent microbial and dust contamination.
- Discard diluted and used solutions along with excess buffer after use.

Procedure for staining paraffin-embedded tissue section slides

- Avoid touching slides and do not allow dust or other debris to contaminate samples, tissues or other material.
- Adjustments can be made to the standard protocol when antigen concentrations are too high or low. Higher antigen concentrations will require less incubation time to obtain optimal staining. Incubation temperature can be increased to 37°C to shorten incubation times.
- Use acid-cleaned slides. Do not use egg albumin dipped slides because trace avidin present in the albumin may cause background.

Materials required

- Phosphate-buffered Saline (PBS): 0.1M phosphate, 0.15M NaCl, pH 7.5 (e.g., Thermo Scientific™ BupH™ Phosphate Buffered Saline Pack, Product No. 28372)
- Blocking Buffer: Add 3 drops of normal serum from the ABC Staining Kit to 10mL of PBS. Alternatively, 1% BSA in PBS may be used.
- Primary Antibody: Adjust to appropriate concentration with Blocking Buffer.
- Biotinylated Secondary Antibody: Add 3 drops (135µL) of normal serum and 1 drop (45µL) of the Biotinylated Secondary Antibody from the ABC Staining Kit to 10mL of PBS.
- ABC Reagent: Prepare reagent 30 minutes before use. Add 2 drops (90µL) of Reagent A to 10mL of PBS followed by 2 drops (90µL) of Reagent B. Immediately mix the solution and allow 30 minutes for the complex to form.
- Detection Substrate such as Thermo Scientific™ Metal Enhanced DAB Substrate Kit (Product No. 34065)

Method

1. Prepare paraffin-embedded tissue section slides according to standard protocols.
2. Wash the paraffin-embedded tissue section with ultrapure water for 5 minutes.

   Note: Endogenous peroxidase activity can be quenched by incubating tissue for 30 minutes in 0.3% H_2O_2 in methanol.
3. Wash slide for 20 minutes with PBS.
4. Block the slide for 20 minutes with Blocking Buffer.
5. Blot excess blocker from the tissue sections. Apply the Primary Antibody and incubate tissue for 30 minutes.
6. Wash slide for 10 minutes with PBS. Apply the Biotinylated Secondary Antibody and incubate tissue for 30 minutes.

   Note: The ABC Reagent may be prepared during this incubation step (see "Materials required" on page 1).
7. Wash slides for 10 minutes with PBS. Incubate the tissue section with the ABC Reagent for 30 minutes.

   Note: Thick sections may require longer staining times.
8. Wash slides for 10 minutes with PBS. Incubate the tissue section with the appropriate substrate.
9. Wash slide for 5 minutes with PBS. Counterstain if desired.

   Note: The time required to perform the above protocol may be reduced with a slight loss in sensitivity by performing the following alterations:

   - Shorten the incubation steps with the primary antibody, the biotinylated secondary antibody and the ABC Reagent to 10 minutes each.
   - Rinse tissue with a gentle stream of ultrapure water instead of the 10 minutes wash after each incubation step.
   - Because the primary antibody is prepared in Blocking Buffer, the incubation steps with the Blocking Buffer and the Primary Antibody may be combined.
   - Increase the concentration of the primary antibody and decrease the incubation times to 2 minutes. Optimization of the concentrations may be required.

Counterstaining

After staining tissue with the ABC Staining Kit, a counterstain may be useful for improving the contrast between the background and the colored reaction products. Two main considerations in the choice of a counterstain are the color of the dye and the pH characteristics of the dye. An acidic stain produces an anion (negatively charged) chromophore upon dissociation. Conversely, a dye is a basic stain if its chromophore is a positively charged ion. Basic dyes have an affinity for the relatively acidic components of tissue, such as DNA, RNA and the nucleus. Acidic dyes preferentially stain the basic constituents of tissue, such as the cytoplasm.

General procedure for Western blotting

Materials required

- Tris-buffered Saline (TBS): 25mM Tris, 150mM NaCl, pH 7.2 (e.g., BupH™ Tris Buffered Saline Packs Product No. 28379)
- Blocking Buffer: Add 3 drops of the normal serum from the ABC Staining Kit to 10mL of TBS. Alternatively 1% crystalline grade BSA in TBS may be used.
- Wash Buffer: TBS, 0.1% BSA and 0.05% Tween™ 20 Detergent
- Primary Antibody: Adjust to appropriate concentration with Wash Buffer (approximately 1-5µg/mL).
- Biotinylated Secondary Antibody: Add 3 drops (135µL) of Blocking serum and 1 drop (45µL) of the Biotinylated Secondary Antibody from the ABC Staining Kit to 10mL of TBS.
- ABC Reagent: Prepare reagent 30 minutes before use. Add 2 drops (90µL) of Reagent A to 10mL of TBS followed by 2 drops (90µL) of Reagent B. Immediately mix the solution and allow 30 minutes for the complex to form.
- Detection Substrate: For chemiluminescent detection, use Thermo Scientific™ SuperSignal™ Substrates such as SuperSignal™ West Pico (Product No. 34080). Depending on the amount of target, colorimetric substrates, such as Thermo Scientific™ 1-Step™ TMB-Blotting (Product No. 34018), may also be used. See the catalog or web site for a complete listing of available substrates.

Method

Note: Perform the following protocol at room temperature. Always use sufficient reagent to cover the entire membrane. Agitate the membrane while performing all wash and incubation steps.

1. After transferring protein to nitrocellulose membrane, place the membrane in flat-bottom dish.
2. Incubate membrane for 1 hour in Blocking Buffer.
3. Rinse membrane three times for 10 minutes each with Wash Buffer. Add Primary Antibody and incubate for 30 minutes.
4. Rinse membrane three times for 10 minutes each with Wash Buffer. Incubate membrane for 30 minutes with the Biotinylated Secondary Antibody.

   Note: The ABC Reagent may be prepared during this incubation step (see "Materials required" on page 2).
5. Rinse membrane three times for 10 minutes each with Wash Buffer. Add the ABC Staining Reagent and incubate membrane for 30 minutes.
6. Rinse membrane three times for 10 minutes each with Wash Buffer.
7. Add the substrate and develop according to manufacturer's recommendations.

General procedure for ELISA

Materials required

- Coating Buffer: 0.2M carbonate-bicarbonate buffer, pH 9.4 (e.g., BupH™ Carbonate-Bicarbonate Buffer Packs, Product No. 28382)
- Phosphate-buffered Saline (PBS): 0.1M phosphate, 0.15M NaCl, pH 7.5 (e.g., BupH™ Phosphate Buffered Saline Pack, Product No. 28372)
- Wash Buffer: 0.05% Tween™ 20 Detergent and 0.1% crystalline BSA in PBS
- Blocking Buffer: Add 3 drops of normal serum from the ABC Staining Kit to 10mL of PBS. Alternatively 1% BSA in PBS may be used.
- Antigen Solution: 10µg/mL in Coating Buffer
- Primary Antibody: Adjust to appropriate concentration with Blocking Buffer
- Biotinylated Secondary Antibody: Add 3 drops (135µL) of the normal serum and 1 drop (45µL) of the Biotinylated Secondary Antibody from the ABC Staining Kit to 10mL of PBS
- ABC Reagent: Prepare reagent 30 minutes before use by adding 2 drops (90µL) of Reagent A to 10mL of PBS followed by 2 drops (90µL) of Reagent B. Immediately mix solution and allow 30 minutes for the complex to form.
- Substrate and substrate stop solution such as 1-Step™ Turbo TMB (Product No. 34022) and 1-2M sulfuric acid

Method

1. Place 100µL Antigen Solution into each well and incubate for 1 hour at room temperature or overnight at 4°C.
2. Empty plate and tap residual liquid on a paper towel. Rinse each well three times with 200µL of Wash Buffer. Empty plate and tap residual liquid on a paper towel.
3. Add 200µL of Blocking Buffer and incubate plate for 1 hour at room temperature.
4. Empty plate and tap residual liquid on a paper towel. Add 100µL of Primary Antibody to each well and incubate plate for 30 minutes at room temperature.
5. Rinse each well three times with 200µL of Wash Buffer. Empty plate and tap residual liquid on a paper towel.
6. Add 100µL of Biotinylated Secondary Antibody to each well and incubate plate for 30 minutes at room temperature.

   Note: The ABC Reagent may be prepared during this incubation step (see "Materials required" on page 3).
7. Rinse each well three times with 200µL of wash buffer. Add 200µL of wash buffer to each well and incubate for 5 minutes.
8. Empty plate and tap residual liquid on a paper towel. Add 100µL of the ABC to each well and incubate for 30 minutes at room temperature.
9. Rinse each well 3 × 200µL with wash buffer. Add 200µL of wash buffer to each well and incubate for 5 minutes. Empty plate and tap residual liquid on a paper towel.
10. Add 100µL of the appropriate enzyme-substrate solution and incubate plate at room temperature for 30 minutes or until sufficient color develops.
11. Add 50µL of the appropriate stop solution (if desired). Measure absorbance at the appropriate wavelength.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Products	Cat. Nos.
Novex™ Tris-Glycine Protein Gels	XP04200BOX
Bolt™ Bis-Tris Plus Protein Gels	NW04120BOX
CL-XPosure™ Film, 5" × 7" sheets, 100 sheets/pkg	34090
SuperSignal™ West Pico Chemiluminescent Substrate, 500mL	34080
SuperSignal™ West Dura Extended Duration Substrate, 100mL	34075
SuperSignal™ West Femto Maximum Sensitivity Substrate, 100mL	34095
Pierce™ ECL Western Blotting Substrate	32106
Pierce™ ECL PLUS™ Western Blotting Substrate	32132
Restore™ Western Blot Stripping Buffer, 500mL	21059
Pierce™ Background Eliminator Kit, for eliminating background from X-ray film	21065

Limited product warranty

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Manufacturer: Pierce Biotechnology, Inc. | Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

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