



ImmunoCruz® rabbit LSAB Staining System: sc-2051

PRODUCT

The ImmunoCruz® rabbit LSAB Staining System includes 15 ml each peroxidase block, serum block (5% normal mouse serum, two 15 ml vials provided), mouse anti-rabbit IgG-Biotin secondary antibody and Avidin D-HRP reagent. Each of these reagents is pre-diluted and ready to use for immunohistochemical staining of formalin-fixed, paraffin-embedded tissue sections. Also included are 1 ml of 50x peroxidase substrate, 1 ml of 50x DAB chromogen and 3 ml of 15x substrate buffer. Number of slides: 150.

Rabbit primary antibody and negative control (rabbit IgG) are provided by the researcher. Rabbit primary antibody should be diluted to 0.5–5 µg/ml, as determined by titration, in serum block (two 15 ml vials provided).

PROCEDURE

ImmunoCruz® LSAB Staining Systems utilize an Avidin D-HRP complex for staining of formalin-fixed, paraffin-embedded tissue sections.

- Clean glass slides with 95% ethanol, treat with subbing solution and air dry. Alternatively, use pre-treated slides.
- Cut 4–6 micron thick tissue sections using microtome and apply to slides. Deparaffinize as follows: 3x xylenes for 5 minutes each, 2x 100% ethanol for 10 minutes each and 2x 95% ethanol for 10 minutes each. Wash in deionized H₂O for 1 minute on stir plate. Aspirate excess liquid from slides.
- Optional: To unmask antigens by heat treatment, place slides in container and cover with 10 mM sodium citrate buffer, pH 6.0. Heat at 95° C for 5 minutes. Top off with fresh buffer and heat at 95° C for 5 minutes (optimal incubation time may vary for each tissue type). Allow slides to cool in buffer for 20 minutes. Wash in deionized H₂O three times for 2 minutes each on stir plate. Aspirate excess liquid from slides.
- All subsequent steps are carried out at room temperature in a humidified chamber. Allow all ImmunoCruz® LSAB Staining System reagents to reach room temperature prior to use. Tissue sections should not be allowed to dry out at any time during the procedure.
- Optional: To quench endogenous peroxidase activity, incubate specimens for 5 minutes in 1–3 drops peroxidase block (white cap). Rinse with PBS and transfer to a PBS wash for 2 minutes on stir plate. Aspirate excess liquid from slides.
- Incubate specimens for 20 minutes in 1–3 drops of serum block (blue cap). Aspirate serum from slides.
- Dilute rabbit primary antibody to 0.5–5 µg/ml as determined by titration. Antibody should be diluted in mouse serum block (provided). Add diluted rabbit primary antibody in sufficient volume to cover the tissue.
- Incubate for 2 hours at room temperature, or overnight at 4° C. Rinse with PBS, then wash in PBS twice for 2 minutes each on stir plate. Aspirate excess liquid from slides.
- Incubate specimens for 1.5 hours in 1–3 drops of biotinylated mouse anti-rabbit IgG secondary antibody (green cap). Rinse with PBS, then wash in PBS twice for 2 minutes each on stir plate. Aspirate excess liquid from slides.
- Incubate specimens for 30 minutes in 1–3 drops of Avidin D-HRP complex (purple cap). Rinse with PBS, then wash in PBS twice for 2 minutes each on stir plate. Aspirate excess liquid from slides.
- During the above incubation step, prepare HRP substrate in the substrate mixing bottle (yellow cap) as follows (sufficient for 15–20 slides): remove tip from mixing bottle and combine 1.7 ml deionized H₂O, 3 drops 15x substrate buffer (orange cap), 1 drop 50x DAB chromogen (yellow cap) and 1 drop 50x peroxidase substrate (yellow cap).
- Add 1–3 drops of HRP substrate to each slide. Develop until light brown staining is visible, usually 30 seconds–10 minutes, although up to 20 minutes may be required. The section may be checked for staining by rinsing with deionized H₂O and viewing under a microscope. If necessary, add additional HRP substrate and continue to incubate. Rinse with deionized H₂O and transfer to deionized H₂O wash for 2 minutes on stir plate.
- Optional: Counterstain slides in Gill's formulation #2 hematoxylin for 5–10 seconds. Immediately wash with several changes of deionized H₂O.
- Optional: Destain with acid alcohol and bluing reagent. Wash with tap water.
- Dehydrate sections as follows: 2x 95% ethanol for 10 seconds each, 2x 100% ethanol for 10 seconds each and 3x xylenes for 10 seconds each. Wipe off excess xylene from edges of slide.
- Immediately add 1–2 drops of permanent mounting medium and cover with glass coverslip. Observe by light microscopy.

STORAGE

Store at 4° C. ****DO NOT FREEZE.**** Stable for one year from the date of shipment.

RESEARCH USE

For research use only; not for use in diagnostic procedures.