SANTA CRUZ BIOTECHNOLOGY, INC.

ImmunoCruz® goat ABC Staining System: sc-2023

PRODUCT

ImmunoCruz® goat ABC Staining System includes 1.0 ml normal mouse blocking serum, 250 µl of 100x mouse anti-goat IgG-Biotin secondary antibody, 0.5 ml each avidin and biotinylated horseradish peroxidase (AB reagents), 1.0 ml 50x peroxidase substrate, 1.0 ml 50x DAB chromogen and 3.0 ml 15x substrate buffer. Also included are mixing bottles for the preparation of reagent working solutions. One ImmunoCruz® ABC Staining System contains sufficient reagent for 200 slides.

Solutions to be provided by the researcher are phosphate buffered saline (PBS) prepared in glass distilled H_2O; 0.1–1% hydrogen peroxide (H_2O_2) diluted in PBS, distilled H_2O or methanol (optional); goat primary antibody; any reagents needed to fix and/or deparaffinize specimens on slides; counterstain (optional); and mounting medium.

PREPARATION OF WORKING SOLUTIONS

Use only freshly prepared buffers. Prepare all working solutions in the mixing bottles provided. After preparation, insert the drop dispenser top (supplied in inverted position) into the cap in correct orientation. Place the entire unit on the bottle and twist the cap. The drop dispenser will snap into place. To remove the drop dispenser for refilling, press laterally with thumb until the top snaps off. To prevent evaporation, secure the caps on bottles when not in use. After completion of the staining procedure, working solutions should be discarded and mixing bottles washed with distilled H_2O.

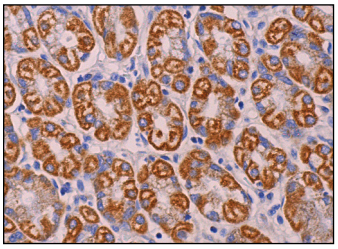
- Blocking serum: In mixing bottle 1 (blue cap), combine 30 µl normal mouse blocking serum stock with 2 ml PBS.
- Biotinylated secondary antibody: In mixing bottle 2 (green cap), combine 30 µl normal blocking serum stock, 2 ml PBS and 20 µl biotinylated mouse anti-goat IgG secondary antibody stock.
- AB enzyme reagent: In AB mixing bottle (purple cap), combine 40 µl reagent A (avidin), 40 µl reagent B (biotinylated HRP) and 2 ml PBS. Mix and let stand for approximately 30 minutes.
- Peroxidase substrate: In substrate mixing bottle (yellow cap), combine 1.7 ml distilled H_2O, 3 drops 15x substrate buffer, 1 drop 50x DAB chromogen and 1 drop 50x peroxidase substrate. Sufficient for 15–20 slides.
- Refer to the Immunoperoxidase Staining Protocol at *www.scbt.com* for tissue section preparation and additional technique notes.

PROCEDURE

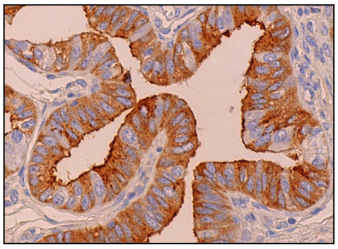
All steps are carried out at room temperature in a humidified chamber. Staining dishes or coplin jars may also be used. Apply sufficient volumes of reagents to completely cover the section; 100 µl is usually adequate, or 1–3 drops of working solutions. Use suction to remove reagents after each step, but avoid drying of specimens between steps.

- Optional: After preparation of tissue sections, slides may be incubated for 5–10 minutes in 0.1–1% hydrogen peroxide diluted in PBS, deionized H_2O or methanol to quench endogenus peroxidase activity. Wash in PBS twice for 5 minutes each.
- Optional: Incubate sections for one hour in 1.5% blocking serum in PBS (mixing bottle 1). This step may be omitted if non-specifc staining is not a problem. Blot excess blocking serum from slides.
- Incubate sections with goat primary antibody for 2 hours at room temperature or overnight at 4° C. Optimal antibody concentration should be determined by titration; recommended range is 0.5–5.0 µg/ml, diluted in 1.5% mouse blocking serum in PBS (from mixing bottle 1). Wash with three changes of PBS for 5 minutes each.
- Incubate sections for 1.5 hours with biotinylated secondary antibody as prepared in mixing bottle 2. Wash with three changes of PBS for 5 minutes each.
- Incubate sections for 30 minutes with AB enzyme reagent (AB mixing bottle). Wash with three changes of PBS for 5 minutes each.
- Incubate sections in 1–3 drops peroxidase substrate (substrate mixing bottle) for 30 seconds–10 minutes or until desired stain intensity develops. The section may be checked for staining by rinsing with H_2O and viewing under a microscope. If necessary, add additonal peroxidase substrate and continue to incubate. Wash sections in deionized H_2O for 5 minutes.
- Optional: Counterstain sections in Gill's formulation #2 hematoxylin for 5–10 seconds. Immediately wash with several changes of deionized H_2O.
- Optional: Destain with acid alcohol and bluing reagent. Wash with tap water.
- For paraffin-embedded tissue sections, dehydrate as follows: 2x 95% ethanol for 10 seconds each, 2x 100% ethanol for 10 seconds each, 3x xylenes for 10 seconds each. Wipe off excess xylenes.
- Immediately add 1–2 drops of permanent mounting medium and cover with a glass coverslip. Observe by light microscopy.

DATA



AIF (N-19): sc-9417. Immunoperoxidase staining of formalin fixed, paraffin-embedded human lower stomach tissue showing cytoplasmic staining of glandular cells. Staining system used: ImmunoCruz® goat ABC Staining System: sc-2023.



Oviductin (N-20): sc-46432. Immunoperoxidase staining of formalin fixed, paraffin-embedded human fallopian tube tissue showing cytoplasmic staining of glandular cells. Staining system used: ImmunoCruz® goat ABC Staining System: sc-2023.

STORAGE

Store all ABC Staining System components at 2–8° 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.