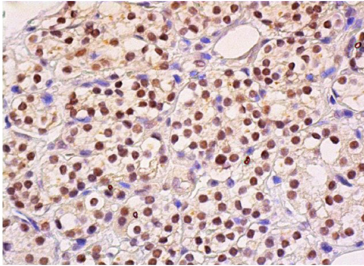
SANTA CRUZ BIOTECHNOLOGY

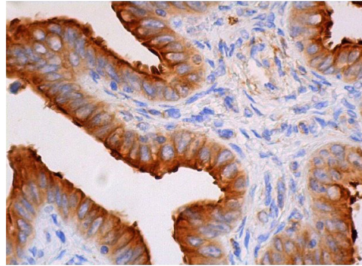
The Power to Question

P R O T O C O L

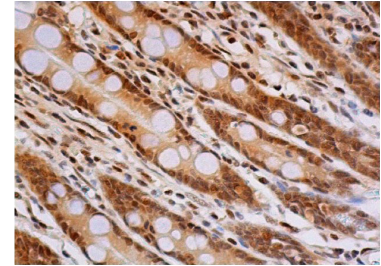
Direct Immunoperoxidase Staining with HRP Conjugates



HuR (3A2) HRP: sc-5261 HRP. Direct immunoperoxidase staining of formalin fixed, paraffin-embedded human parathyroid gland tissue showing nuclear staining of glandular cells.



β Tubulin (D-10) HRP: sc-5274 HRP. Direct immunoperoxidase staining of formalin fixed, paraffin-embedded human fallopian tube tissue showing cytoplasmic and membrane staining of glandular cells.



Ub (P4D1) HRP: sc-8017 HRP. Direct immunoperoxidase staining of formalin fixed, paraffin-embedded human duodenum tissue showing nuclear and cytoplasmic staining of glandular cells.

DIRECT IMMUNOPEROXIDASE STAINING PROTOCOL:

- For direct immunoperoxidase staining of tissue sections, we recommend the use of the *Santa Cruz Biotechnology, Inc. HRP-conjugated primary antibodies.*

- All steps are carried out in a humidified chamber. Allow all staining reagents to reach room temperature prior to use. Tissue sections should not be allowed to dry out at any time during the procedure. Use suction to remove reagents after each step, but avoid drying of specimens between steps. Use sufficient reagents to cover the specimens (approximately 100 µl per slide is usually adequate).

  Optional: Certain antigenic determinants are masked by formalin fixation and paraffin embedding and may need to be exposed by heat, protease, or detergent treatment (Antigen unmasking).

  Optional: Incubate tissue sections for 5–10 minutes in 0.1–1% hydrogen peroxide in deionized H_2O to quench endogenous peroxidase activity. Wash in PBS twice for 5–7 minutes each.

- Incubate tissue sections for 1 hour at room temperature in 5% normal blocking serum in PBS. Blocking serum ideally should be derived from the same species in which the primary antibody is raised. Remove blocking serum from slides.

- Incubate tissue sections with HRP-conjugated primary antibody overnight at 4° C. Optimal antibody concentration should be determined by titration; recommended range is 2.0–8.0 µg/ml diluted in PBS with 5% normal blocking serum. Wash with three changes of PBS for 5–7 minutes each.

- For chromogenic detection, add 1–3 drops HRP substrate mixture (e.g. 3,3'-Diaminobenzidine, or DAB, sc-216567) Develop for 30 seconds–10 minutes, or until desired stain intensity develops. Rinse with deionized H2O and transfer to a deionized H_2O wash for 2 minutes on a stir plate. If desired, counter-stain in Gill's formulation #2 hematoxylin (sc-24973) for 5–10 seconds. Immediately wash with several changes of deionized H_2O.

- Dehydrate through alcohols and xylenes as follows: Soak in 90% ethanol twice for 3 minutes each, then 100% ethanol twice for 3 minutes each, then xylenes three times for 10 seconds each. Wipe off excess xylene. Immediately add 1–2 drops of permanent mounting medium (e.g., Clarion sc-24942), cover with a glass coverslip (sc-24975) and observe by light microscopy.