

NB200-305 Protocol

Immunohistochemistry protocol specific for Estrogen Receptor beta Antibody (NB200-305)

Immunohistochemistry protocol specific for Estrogen Receptor beta Antibody (NB200-305):

https://www.novusbio.com/products/er-beta-nr3a2-antibody-14c8_nb200-305

Immunohistochemistry Procedure

1. Cut 4-5 micron sections and mount onto Superfrost plus slides.
2. Dewax in xylene and rehydrate through graded alcohols.
3. Block with 3% H₂O₂ in methanol for 10 minutes.
4. Wash in running tap water for 5 minutes then block non-specific binding sites with normal horse serum.
5. To unmask the antigen, heat-mediated retrieval is required using either a microwave or pressure cooker. For microwave-mediated retrieval immerse sections in 1 mM citrate buffer, pH 6, and microwaved at full power (600W) for 27 minutes (NB The exact time will depend on both the power and age of the microwave). Alternatively, sections can be pressure cooked for 4 minutes, immersed in 1 mM citrate buffer, pH 6. Allow sections to cool for 20-30 minutes and wash in running tap water for 5 minutes.
6. Incubation sections with 5ug/ml primary antibody at 4C overnight. Incubation time and temperature are critical.
7. Incubate with appropriate biotinylated secondary antibody for 30 minutes at room temperature, followed by streptavidin ABC kit (DAKO) according to the manufacturer's instructions.
8. Prepare 3,3'-diaminobenzidine substrate and apply for 5-10 minutes.
9. Counterstain with haematoxylin, dehydrate and coverslip.

Using this protocol, we detect consistent, strong ER β staining in epithelial cell nuclei. Occasional weak to moderate staining is seen in surrounding stromal and endothelial cell nuclei. Sporadic light cytoplasmic staining is sometimes observed. We have also successfully detected ER β in colon and ovarian tumours using the same antibody.