

Validation notes.

Specificity of antibodies

Our validation process incorporates a variety of steps to ensure the specificity and robustness of the antibody:

Following a thorough search of the literature, we will establish an appropriate positive control tissue for the antigen in question. We normally look at 2-3 commercially available antibodies for a given specificity, and will consider polyclonal as well as monoclonal antibodies. In all cases we will attempt to find an antibody which is known to work on FFPE tissue, as this is usually a requirement of the client.

A variety of antigen retrieval methods, including pressure cooking and microwave will be attempted, although we try to avoid the use of proteolytic enzymes as these present the greatest problem in terms of reproducibility.

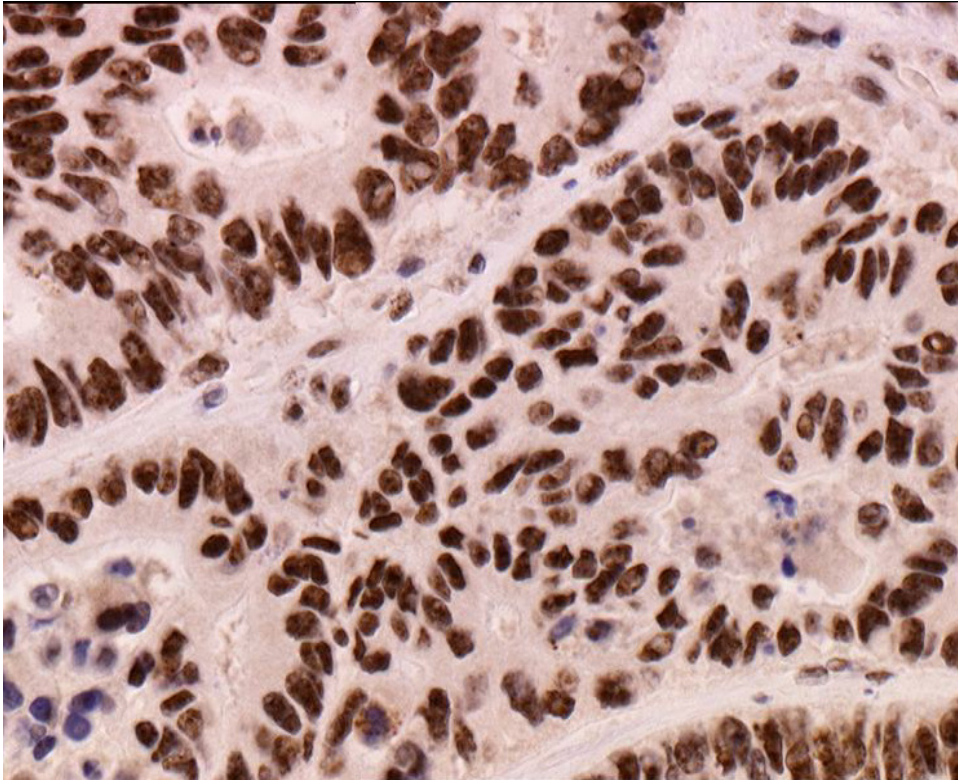
Other parameters such as incubation time and antibody concentration will be examined on the bench, with the aim of translating the method as quickly as possible to the Ventana Discovery or Dako Autostainer platforms.

Detection is routinely by the ABC-peroxidase method, with DAB as the chromogen and haematoxylin as counter-stain. The majority of our automated image analysis protocols are optimised for this colour range.

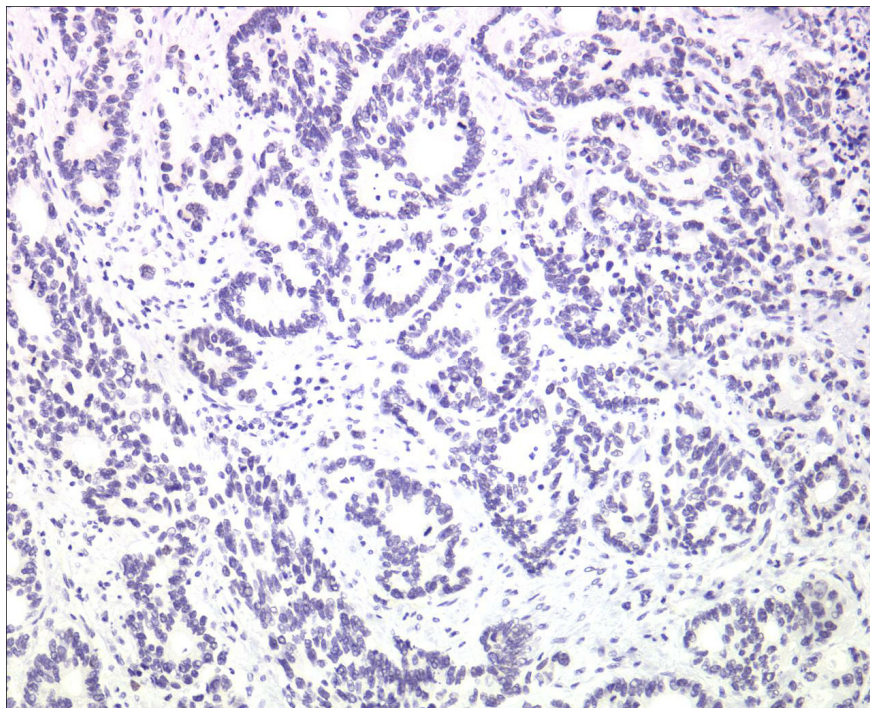
Specificity is controlled in a number of ways. In all cases the staining pattern is compared with published literature, and the pattern is evaluated by a qualified pathologist to confirm its correctness. Further controls include the use of blocking peptides (if available). For antibodies against phosphoproteins we routinely use prior dephosphorylation with alkaline phosphatase as a control to confirm the specificity of the antibody. **Figs 1-3** shows the staining of a tumour with antibody against phospho-pRb, the product of an important tumour suppressor gene. The images show that blocking with peptide or pre-treatment of the section with alkaline phosphatase completely eliminates staining, confirming the excellent specificity of this antibody and the test.

Figs 1-3

PRb in Breast cancer



Phosphatase Treatment.



Peptide Block

