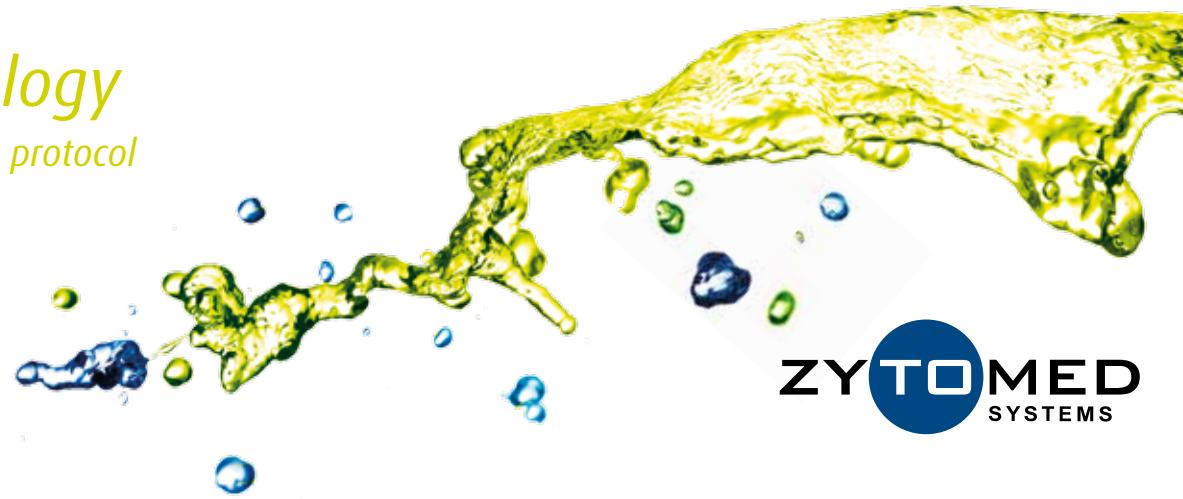
Immunohistology

Sequential double stain protocol



ZYTOMED SYSTEMS

Vimentin (clone V9)
without pre-treatment

Vimentin (clone V9) treated
with HIER pH 6.0

PIN Cocktail (p63 + p504S)
treated with HIER pH 6.0

PIN Cocktail (p63 + p504S)
treated with HIER pH 6.0

How to establish a new antibody in immunohistochemistry

Before using a new antibody or IHC in the day-to-day routine of your lab it is reasonable to evaluate the optimal staining protocol for this particular antibody. For the first protocol use the guidelines in the data sheet. Usually the manufacturer makes recommendations for the following two important points:

- Pre-treatment of FFPE tissue sections
- Dilution of the new antibody

These two parameters are critical for obtaining an optimal staining result in immunohistochemistry. Other important parameters are the sensitivity of the detection system, the chromogenic substrate and the device used for pre-treatment.

When starting to evaluate the new antibody do not modify too many variables at the same time. Use your usual detection system and chromogenic substrate and start with the pre-treatment recommended by the manufacturer. The first parameter you should vary is the dilution of the antibody. When the manufacturer recommends a dilution of 1:50 to 1:100 start the dilution series with 1:25 and dilute further up to 1:500.

The tissue for testing should be a tissue which is known to be positive for the antibody. This positive control is mentioned in the data sheet too. Do not use patient material that may or may not be positive.
It is important for the evaluation to use sections from the same block for the entire evaluation series.

To determine the best pre-treatment method you should start with the method recommended by the manufacturer. However, it is always worthwile to compare heat pre-treatment methods with enzymatic pre-treatment. Some antibodies also show good results without any pre-treatment.
By the way: a term often used in connection with pre-treatment is HIER which means Heat Induced Epitope Retrieval.

When there is no dilution or pre-treatment given in the data sheet the dilution can be calculated from the immunoglobulin concentration of the antibody. Usually, the antibody concentration in the working solution is between 0.1 and 10 µg/ml. Thus, a concentrated antibody with an immunoglobulin concentration of 1 mg/ml should be diluted from 1:100 up to 1:10000 in the initial tests.

In order to review the staining results it is important to know the correct staining pattern of the antibody at cellular level. This means you should know wether the antibody stains the cytoplasmic membrane, the cytoplasm or the nucleus of the cell before you evaluate the results.

A strong specific signal with no or only very slight background staining is optimal.

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