Protocol

Antigen Retrieval Techniques for use with Formalin-Fixed Paraffin-Embedded Tissues

IHC7

In many cases the fixation and processing steps involved in the preparation of tissue results in loss of antigen immunoreactivity. Often this can be reversed by using appropriate antigen retrieval techniques such as microwave antigen retrieval or proteolytic digestion.

Standard protocols for some of these techniques are outlined below. It should be noted that in many cases, incubation times will vary depending upon the particular processing method used, the antigen to be retrieved and/or the antibody being used.

This method provides a general procedure for use with the majority of Bio-Rad reagents. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with product and batch specific information provided with each vial. Please note that a certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques - these are guidelines only and may need to be adjusted for particular applications.

These techniques can be used with the following protocols:

- Indirect Immunostaining of Paraffin-Embedded Tissue Sections
- Streptavidin-Biotin Immunostaining of Paraffin-Embedded Tissue Sections
- PAP/APAAP Immunostaining of Paraffin-Embedded Tissue Sections

Proteolytic antigen retrieval using trypsin

Reagents:

- Calcium Chloride, ($CaCl_2$), 0.1 g
- Trypsin (Sigma Type II), 0.1 g
- Distilled Water, 100 ml
- Sodium Hydroxide, (NaOH), 0.1 M

Method:

1. Dissolve $CaCl_2$ in distilled water and adjust the pH to 7.8 with NaOH. Store at 37°C.
2. Dissolve trypsin in the $CaCl_2$ solution.
3. Place sections in trypsin solution at 37°C and incubate for a pre-determined optimum time (approximately 20-30 minutes).
4. Wash in TBS and proceed with staining.

Proteolytic antigen retrieval using pronase (PIER)

Reagents:

- Calcium Chloride, ($CaCl_2$), 0.1 g
- Pronase 0.1 g
- Distilled Water, 100 ml
- Sodium Hydroxide, (NaOH), 0.1 M

Method:

1. Dissolve $CaCl_2$ in distilled water.
2. Dissolve pronase in $CaCl_2$ solution and pH to 7.8 with NaOH.
3. Place sections in pronase solution at room temperature and incubate for pre-determined optimum time (approximately 10 minutes).
4. Wash in TBS and proceed with staining.

Heat-induced epitope retrieval (HIER)

Heat-mediated antigen retrieval may be used in many situations to enable staining of certain antigens in paraffin-embedded tissue sections, or sometimes to improve results when staining is weak or inconsistent.

A number of buffers may be used for this purpose. The precise choice is usually recommended on product specific datasheets, or determined experimentally. For your convenience, Bio-Rad offers a range of pre-prepared antigen retrieval buffers at different pH levels guaranteeing consistency of performance and reliability. Alternatively, buffers may be prepared using the two recipes below. Please contact Bio-Rad's Technical Services Department for more details.

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Recipe for 10 mM citrate buffer for antigen retrieval

For a 20x stock solution

- Citric acid, 10.5 g
- Distilled water, 250 ml

For a 1x solution

- 20x Stock Solution, 25ml Distilled water, 475 ml
- Adjust to pH 6.0 with 1 M NaOH

Recipe for 10 mM Tris 1mM EDTA buffer for antigen retrieval

- Disodium EDTA dihydrate, 0.37 g
- Tris base, 1.21 g
- Distilled water, 1000 ml
- Adjust pH to 9.0 with 1 M HCl

Method:

1. Place slides in 500 ml of buffer (use a microwavable container).
2. Microwave on high for 15 minutes without a lid, or cover with vented cling-film and heat at 90°C for 10 minutes (buffer must not boil).
3. Let sections stand for 15 minutes to cool.
4. Wash in TBS and proceed with staining protocol.

Please note that the actual heating times may vary depending on the antibody and the fixation processes being used.

Are you interested in learning more about which reagents to use in your immunohistochemistry experiments? Bio-Rad's immunohistochemistry application page enables you to quickly and easily find antibodies, kits and reagents tested in immunohistochemistry.
Find out more at: bio-rad-antibodies.com/ihc-resources