

Worksheet IH7

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

(For use with Worksheets IH4, 5 and 6)

In many cases the fixation and processing steps involved in the preparation of tissues results in loss of immunoreactivity of antigens. Often this can be reversed by the use of 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 that has been used, or for different antigens.

Note:

This method provides a general procedure for use with the majority of Biozol 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.

Proteolytic antigen retrieval using Trypsin

Reagents:

Calcium Chloride	0.1g
Trypsin (Sigma Type II)	0.1g
Distilled Water	100 ml
Sodium Hydroxide (0.1 M)	

Method:

1. Dissolve calcium chloride in distilled water and pH to 7.8 with sodium hydroxide. Store at 37°C.
2. Dissolve trypsin in calcium chloride solution.
3. Place sections in Trypsin solution at 37°C and incubate for pre-determined optimum time (approximately 20-30 minutes).
4. Wash in TBS and proceed with staining.

Proteolytic antigen retrieval using pronase

Reagents:

BIOZOL

Diagnostica Vertrieb GmbH
Obere Hauptstr. 10b
D-85386 Eching
Postfach 2022
D-85380 Eching

.. freecall **Bestell-Tel.: 08000-BIOZOL**
(entspricht der Tel.-Nr. 0800-024 69 65)

Tel.: 089-37 99 666-6
Fax.: 089-37 99 666-99
info@biozol.com
www.biozol.com

Calcium Chloride	0.1g
Pronase	0.1g
Distilled Water	100 ml
Sodium Hydroxide (0.1 M)	

Method:

1. Dissolve calcium chloride in distilled water.
2. Dissolve pronase in calcium chloride solution and pH to 7.8 with sodium hydroxide.
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 mediated antigen retrieval

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

A number of buffers may be used for this purpose. The choice of buffer should be as recommended on product specific datasheets, or determined experimentally by the customer. Biozol offer a range of pre-prepared antigen retrieval buffers, or alternatively customers may prefer to prepare their own buffers using the recipes provided below.

Reagents

Antigen retrieval buffer

Method:

1. Place slides in suitable container of prepared buffer, cover with cling-film (vented) and heat to 90°C (Please note buffer must boil). Keep at this temperature for 10 minutes
2. Let sections stand for 15 minutes to cool.
3. Wash in TBS/tween and proceed with staining.

Note:

Actual heating times may vary depending on the antibody being used and the fixation processes that have been utilised.

BIOZOL

Diagnostica Vertrieb GmbH
Obere Hauptstr. 10b
D-85386 Eching
Postfach 2022
D-85380 Eching

.. freecall **Bestell-Tel.: 08000-BIOZOL**
(entspricht der Tel.-Nr. 0800-024 69 65)

Tel.: 089-37 99 666-6
Fax.: 089-37 99 666-99
info@biozol.com
www.biozol.com

Solutions used:

TBS (Tris buffered saline) (stock solution x10 concentrated)

Sodium chloride 87.66 g
Tris 60.55 g
Distilled water 1 litre
Adjust pH to 7.4 using concentrated HCl.

TBS/Tween

Working strength TBS 1 litre
Tween 20 0.5 ml

0.01M Citrate buffer for antigen retrieval

Anhydrous citric acid 3.84 g
Distilled water 1800 ml
Adjust pH to 6.0 using concentrated NaOH.
Make up to 2 litres with distilled water

0.001M EDTA buffer for antigen retrieval

EDTA 0.37 g
Distilled water 1000 ml
Adjust pH to 8.0 with 1 M NaOH

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE

BIOZOL

Diagnostica Vertrieb GmbH
Obere Hauptstr. 10b
D-85386 Eching
Postfach 2022
D-85380 Eching

.. freecall **Bestell-Tel.: 08000-BIOZOL**
(entspricht der Tel.-Nr. 0800-024 69 65)

Tel.: 089-37 99 666-6
Fax.: 089-37 99 666-99
info@biozol.com
www.biozol.com