



DIMENSION LABORATORIES INC.
12 FALCONER DRIVE, UNIT 4
MISSISSAUGA, ONTARIO L5N 3L9
PHONE - TORONTO (905) 858-8510
ONTARIO & QUEBEC 1-800-668-4630
ALL OTHER PROVINCES 1-800-387-8257
FAX (905) 858-8801

PROTOCOL SHEET

HIGH TEMPERATURE EPITOPE UNMASKING

The following protocols are suggestions for high temperature epitope unmasking (HIER). Many antibodies require such treatment in order to uncover epitopes masked by formalin fixation. In the case of other antibodies, high temperature unmasking can take the place of enzyme digestion. Individuals can use the following suggestions to determine their own working procedures. Individual pressure cooking and microwave pressure cooking protocols are available on request.

1) Deparaffinize sections and rehydrate to water.

2) Dilute DIMENSION Retrieval Solution concentrate (D2200S-100ML or D2210S-100ML) 1:10 in deionized water (DIW). Make up 50 ml for each coplin jar to be used, or enough to fill the retrieval vessel (see alternate methods).

3) Place 50 ml of the retrieval solution in each coplin jar. Heat the fluid (85-95° C) then add the slides, and carry out high temperature epitope unmasking using the pressure cooker, the microwave, or the water bath method as described below. It is the **application of heat** which causes the unmasking of the epitopes. The pressure cooker is the most highly recommended protocol, but the technique which best suits the individual laboratory should be used.

a. Pressure Cooker/ Autoclave

Fill the pressure cooker to a 5 cm depth with water and bring it to a boil. Place the coplin jars into the pressure cooker, then add the slides when the buffer is hot. Fit the caps on the jars loosely, then close the lid of the pressure cooker, and lock it. When the pressure indicator valve has risen after about 4 minutes, incubate the sections for 2 to 5 minutes. Remove the pressure cooker from the heat source, and run under cold water. Open the lid **ONLY WHEN THE PRESSURE VALVE SINKS**. (a protocol which does not make use of coplin jars is on the reverse side of this sheet) Continue with step 4.

b. Microwave Oven

Cover the jars filled with the slides and the retrieval solution using cling wrap (vented with a small hole). An elastic band may be placed around the neck of the jar to keep the plastic on during heating. The jars should be placed at precise locations in the microwave which are marked so that

the same locations are used in the treatment of future slides. A microwave oven with a rotating base is most desirable. A constant number of jars should be used each time the protocol is used. When only a small number of slides are being treated, fill the other jars up with water and blank slides. Heat in a 650-800 W microwave oven for two 5 minute cycles at full power. Top up the fluid with warm deionized water to replace any lost fluid after the first five minutes. It is important to keep as much consistency as possible between different treatments with the microwave. Continue with step 4.

c. Hot Water Bath

Set up a hot water bath (95-99° C) by placing water into a large vessel such as a kitchen pot on a hot plate. Place polyethylene coplin jars filled with the retrieval solution into the hot water bath and warm the buffer to 95-90° C. Place the slides into the solution and then cover the jars loosely with the caps. Incubate the slides for 30 minutes at 90-95° C then remove the coplin jars from the water bath. Continue with step 4.

- 4) Eliminate the heat source and let the sections stand in the warm solution for 20 minutes.
- 5) Gently rinse the slides with buffer (TBS or PBS pH 7.6) for five minutes, then wash in DIW.
- 6) Begin established staining procedure.

ALTERNATE PROCEDURES - Pressure cooking without the use of coplin jars:

If a high number of slides are being treated you may put enough retrieval solution to cover the slides directly into the pressure cooker, and immerse the staining racks into the retrieval solution. The same is true for the water bath.

OPTIMISING THE RETRIEVAL TIME:

Timing should be optimised in each laboratory. Some labs report optimum staining after 2 minutes of pressure cooking, and that a loss of morphology occurs beyond this time. Slides placed on a rack directly into the heating vessel rather than in a coplin jar will require less time. In the microwave technique timing will vary according to the power output of the oven.

PRESSURE COOKING IN THE MICROWAVE OVEN:

A microwaveable pressure cooker (D0300X) is now available from DIMENSION LABORATORIES. Once full pressure is achieved with this apparatus, the heating should continue for 2 to 5 minutes. One litre of retrieval solution is sufficient to cover the slides in a plastic staining rack laid out on the mesh bottom of the pressure cooker. Once heating is complete the pressure cooker should be left to sit in the oven for 20 minutes so that the solution can cool and the pressure can decrease.

Autoclaves can be used at 120°C, 15 psi, for 10 minutes. Sections are then cooled in water and transferred to buffer.



Place orders with:
DAKO Diagnostics Canada Inc.
12 Falconer Dr, Unit 4
Mississauga, Ontario, Canada
L5N 3L9

PH: (905) 858-8510 FAX: (905) 858-8801
Ont. & Que. 1-800-868-4630
Other Prov. 1-800-387-8257