

Declere™

For In Vitro Diagnostic Use (IVD)
Instructions for use

INTENDED USE

Declere™ is intended for the use in immunohistochemistry (IHC) staining protocols. This reagent is designed to pretreat formalin fixed, paraffin embedded tissue sections.

SUMMARY AND EXPLANATION

Declere™ is a product that combines the three pretreatment steps: deparaffinization, rehydration, and unmasking in immunohistochemistry stains. Use of this product standardizes the pretreatment procedure.

PRINCIPLES AND PROCEDURES

Pretreatment buffers are used to prepare specimens for immunohistochemical staining protocols. This solution helps maintain the morphological characteristics of the tissue while preparing epitopes for specific binding of antibodies within an immunochemical reaction.

MATERIALS AND METHODS

Reagents Supplied As:

Reagent Cat. No.	Contents	Vol. (ml)
921P-04	Concentrated 20X	50.0 mL
921P-06	Concentrated 20X	200.0 mL
921P-09	Concentrated 20X	1000.0 mL

Materials and Reagents Needed But Not Provided

- | | |
|--|--------------------------------|
| 1. Primary Antibody(ies) | 9. Slide rack* |
| 2. TBS or PBS wash buffer* | 10. Staining dishes* |
| 3. Volumetric flask/graduated cylinder | 11. Pressure cooker* |
| 4. Microscope slides, positively charged | 12. Proteolytic enzyme |
| 5. Drying oven | 13. Peroxidase block |
| 6. Positive and negative controls | 14. Negative control reagents* |
| 7. Clearing agent (xylene, Clearene, etc.) | 15. Detection kits* |
| 8. Ethanol or reagent alcohol | 16. Chromogen* |
| | 17. Hematoxylin* |
| | 18. Mounting medium |

*See Cell Marque catalog for product numbers. Some of the reagents listed are based on specific application and detection system used.

Storage and Stability

Store at 20-26°C, up to 36 months from the date of manufacture (see product label for expiration date).

Reagent Preparation

For working solution, dilute concentrated Declere™ with deionized water 1:20. You also may dilute the entire 200.0 mL of reagent to make up to 4 liters of working solution of Declere™.

Recommended Protocol(s)

One-Step Procedure

- Cut 3-4 micron sections and place on positively charged slides. Air Dry.
- Dry sections in incubator for at least 2 hrs. at 58° C.
- Place up to 24 slides in plastic slide rack.
- Place slide rack into staining dish and fill with enough working solution of Declere™ so that all tissues on the slides are submerged. Prepare a second staining dish filled with approx. 200 ml of working solution of Declere™ (This will be used as a hot rinse following processing time in the first staining dish). If using an electric pressure cooker for HIER, make sure that the staining dishes do not press against the pressure pin when viewed from the underside of the lid of the pressure cooker. Should this occur, there will be interference with the locking mechanism and make it difficult to unlock and open the lid following completion of the pressure cycle.
- Various heating methods may be used; however, the pressure cooker method often produces more consistent results.
- Electric Pressure Cooker Method: Place approx. 700 ml of water in the base of unit and set inverted (i.e. legs pointing upward) rack into the water. Put both staining dishes inside the pressure cooker. Lock pressure cooker lid in place atop the pressure cooker and make sure vent switch located on the lid handle is in the closed position. Make sure the pressure weight is rotated until it is completely seated in its receptacle otherwise the pressure cooker will not be able to build pressure. Using the buttons on the control panel, set pressure mode for "high" and move "up" arrow until you reach 15 then press the "start" button.
- Timer will start to count down when the correct pressure and temperature are reached.
- After timer goes to "zero", push "off" button.
- Wait approximately 5 minutes then move the vent switch away from the closed position so as to release pressure. Red pin atop lid will descend when all the pressure is released so you can safely remove the lid. Simply press the red button located on the base of the lid handle while rotating the lid to the open position.
- Transfer slides from first container to the hot rinse (second container) using a forceps to lower the slide rack very slowly into the hot rinse solution. Caution: Since the hot rinse solution is superheated, rapid immersion of slide rack may cause sudden boil out of the hot solution.
- Agitate slides and let sit for a maximum of 2 minutes (first solution can be discarded and you may reuse the hot rinse solution in the first container next time the procedure is performed.)

12. Rinse slides in PBS or TBS IHC wash buffer and proceed with IHC protocol.
13. Wash Declere™ off with PBS or TBS IHC wash buffer. The Declere™ in the staining dish into which slide rack was initially placed must now be discarded. The Declere™ in the second (hot rinse) staining dish may be reused as your initial Declere™ next time you repeat the process i.e. you may rotate the solutions once.
14. Continue IHC staining according to procedure routinely employed.

Three-Step Procedure

1. Cut and dry your paraffin sections as described above then deparaffinize and rehydrate your sections.
2. Place slide rack into staining dish and fill with enough working solution of Declere™ so that all tissues on the slides are submerged.
3. Electric pressure cooker method (preferred method): follow steps 6-12 above except use only one container of Declere™. After pressure cooker lid has been removed, allow slides and container to remain in place for a maximum of 2 minutes then wash Declere™ off with PBS or TBS IHC wash buffer.
4. Continue IHC staining according to procedure routinely employed.

INTERPRETATION OF RESULTS

The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

QUALITY CONTROL PROCEDURES

Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM4-A Vol.19 No.26 for more information on tissue controls.

WARNINGS AND PRECAUTIONS

1. This product is for *in vitro* diagnostic use by professionals only.
2. Do not use after expiration date printed on product labels. The user must validate any storage conditions other than those specified in the package insert.
3. Bring all reagents, slides, and specimens to room temperature (18-24° C) prior to use.
4. Cross contamination of reagents or samples may give false results.
5. Avoid microbial contamination of reagents, as this could produce incorrect results.
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.

8. Avoid splashing or generation of aerosols at all times.
9. Reusable glassware must be washed and thoroughly rinsed free of detergents prior to use. All glassware must be clean and dry before use.
10. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.
11. Refer to product SDS.
12. Always check to make sure that pressure drops back to normal before opening a device under pressure.
13. Always wear personal and protective equipment to avoid contact with high heat solutions.
14. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts, loss of tissue, false positive or false negative results.

LIMITATIONS

Immunohistochemistry is a multi-step process that is dependent on the pre-analytical variables involved in specimen processing prior to IHC staining. It is the responsibility of the end user to determine optimal conditions.

TROUBLESHOOTING

Refer to reagent-specific protocol recommendation according to data sheet provided.

For further help, feel free to contact Cell Marque's Technical Support at +1-800-665-7284.

REFERENCES

1. NCCLS Quality Assurance for Immunocytochemistry approved guideline, December 1999 MM4-A Vol. 19 No.26 for more information on tissue controls.

DISCLAIMERS



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