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DATA SHEET

Antigen Retrieval *AR-10* Solution

Cat. Nos. HK057-5K, HK058-5K & HK058-20K

Doc. No. HK058, Rev. No.G
Date of Release: 11-Nov-2022

REAGENTS SUPPLIED

Store at 2-8°C

One of the following:

HK057-5K:	One bottle (100 ml), 10X concentrate, Antigen Retrieval <i>AR-10</i> Solution
HK058-5K:	One bottle (250 ml), ready-to-use, Antigen Retrieval <i>AR-10</i> Solution
HK058-20K:	One bottle (1000 ml), ready-to-use, Antigen Retrieval <i>AR-10</i> Solution

Intended Use

This product may be used to recover antigenicity in formalin-fixed, paraffin-embedded tissue. This product is a General Purpose Reagent, suitable for diagnostic histopathology, laboratory and research use.

Description

The Antigen Retrieval technique[†] is a novel method for the rescue of antigens from formalin-fixed, paraffin-embedded tissue. It consists of heating sections in a microwave oven in the presence of an antigen retrieval solution.

The quality of the staining result is largely dependent on **strict adherence** to the antigen retrieval protocol. If the antigens are incompletely retrieved, the staining is light and the background may be high. A user following the standard protocol outlined on reverse side will see crisp, clear nuclear staining with little or no background.

Limitations

The antigen retrieval protocol is recommended for use with tissues fixed *with formalin only*. Other fixatives or fixation procedures may not produce comparable results. Interpretation of the staining results is solely the responsibility of the user.

Preparation of Reagents

Antigen Retrieval *AR-10* Solution. HK058-5K, is ready to use. Antigen Retrieval *AR-10* Solution, 10X HK057-5K, is concentrated solution and should be diluted tenfold with deionized water (mix one part of concentrated Antigen Retrieval *AR-10* Solution with nine parts of deionized water).

References

1. Shi, S.R., et al. Antigen Retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem* 39:741, 1991.
2. Gown, A.M., et al. Microwave-based antigenic unmasking: a revolutionary new technique for routine immunohistochemistry. *Appl Immunohistochem* 1:256-266, 1993.
3. Shi, S.R., et al. Antigen Retrieval technique: a novel approach to immunohistochemistry on routinely processed tissue sections. *Cell Vision* 2:6-22, 1995.
4. Shi, S.R., et al. Antigen Retrieval immunohistochemistry under the influence of pH using monoclonal antibodies. *J Histochem Cytochem* 43:193-201, 1995.

Purchase of this product includes permission to practice U.S. patents No. 5,244,787 and 5,578,452 for non-commercial and non-reimbursable testing only. Please contact BioGenex for information regarding a license for any other use.

[†]The Antigen Retrieval technique is covered by BioGenex's U.S. Patents No. 5,244,787 and 5,578,452.

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Antigen Retrieval AR-10 Solution

Cat. Nos. HK057-5K, HK058-5K & HK058-20K

General Purpose Reagent suitable for diagnostic Histopathology, laboratory and research use.

Standard Protocol

1. Rinse slides in deionized water. Place slides in a plastic staining holder** with any empty slots filled with blank slides. Place the holder in a white slide bath containing 250 ml of working strength Antigen Retrieval AR-10 Solution. Place the lid loosely on the bath and center it inside a microwave oven on a paper towel to adsorb any liquid runover.
2. Turn the oven on high power (500-1,000 watts) and closely watch the solution until it comes **to a rapid boil**, and then turn off the oven. (Note: It usually takes 3-7 minutes before a boil is reached. However, the amount of time required may vary significantly depending on a number of factors, such as the starting temperature of the retrieval solution, the wattage of the microwave oven, the age of the oven and the inside temperature of the oven. It is very important that a rapid boil is reached for every run before proceeding to the next step.)
3. Set oven power to approximately 50% level and heat for 10 to 15 minutes. (Note: The power setting should be adjusted so that the oven cycles on and off every 20-30 seconds and the solution boils about 5-10 seconds each cycle. A successful run should give frequent, but not continuous boiling without any liquid runover. This power setting should be noted and used for this step in all subsequent runs for the same antibody. Each antibody should be tested for the optimal time for this step.)
4. Remove the slide bath from the microwave oven. Allow slides to cool for 20-30 minutes at room temperature. Rinse with several changes of deionized

water. Place slides in 1X PBS and continue with the immunostaining procedure.

Alternate Method: Microwave Pressure Cooker Protocol

The alternate method uses a pressure cooker inside of the microwave. The pressure cooker that we recommend is available through Nordic Ware®, or you may purchase it through BioGenex for your convenience* .

1. Place slides in a slide holder** filling empty slots with blank slides. Put the holder in the white slide bath containing 250 ml of working strength Antigen Retrieval AR-10 Solution. Put the lid loosely on the bath (placed at an angle to permit release of pressure).
2. Fill a plastic pressure cooker with 600 ml of distilled water. Place three baths side-by-side in the pressure cooker and cover the pressure cooker according to the manufacturer's instructions.
3. Center the pressure cooker inside the microwave oven, and turn the oven on high power (800-850 watts) for about 15 minutes, until the cooker is fully pressurized.
4. Reduce the microwave power level to 300-350 watts (40% power for 800 watt ovens) and allow the solution to simmer for 15 minutes. Then remove the pressure cooker from the microwave oven and carefully open the cover according to the manufacturer's instructions. Leaving the slides in the cooker, allow the slides to cool for 20 minutes, then continue with the immunostaining procedure.

* Microwave Tender Cooker®, Cat. No. 62104, Nordic Ware®, Minneapolis, MN; BioGenex Cat. No. NW001-PC.

** The microwave Antigen Retrieval accessory kit (MW001-HB) contains 3 sets of slide baths, lids and holders. These are similar to the Tissue Tek® gray slide holders for 22 slides and the WHITE staining dish with lid which will also work well with these protocols.

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