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DATA SHEET

EZ -AR™ Solutions

Recommended for use with the EZ-Retriever®

Doc. No. 932-HK521, Rev. No. S
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INTENDED USE

The EZ-AR™ solutions are designed to deparaffinize, rehydrate and recover antigenicity of epitopes in formalin-fixed, paraffin-embedded tissue sections. These products are for Laboratory Use Only.

DESCRIPTION

EZ-AR™ Common

1. EZ-AR™ Common performs dewaxing and rehydration in formalin-fixed, paraffin-embedded tissue sections using microwave heating. It is non-toxic, non-flammable and odorless. EZ-AR™ Common can be used alone or in combination with EZ-AR™ 1, EZ-AR™ 2, AR Ultra Solution depending on antigen retrieval requirements.

EZ-AR™ 1, EZ-AR™ 2, AR Ultra Solutions:

These solutions perform antigen retrieval in formalin-fixed, paraffin-embedded tissue sections using microwave heating. Different tissues require different types of pretreatment conditions. Depending on the nature of the tissue and the antigen retrieval requirement, one of the EZ-AR™ solutions may be used. EZ-AR™ 1 is Citra-based solution; EZ-AR™ 2 and AR Ultra Solution are an EDTA based solutions. The use of EZ-AR™ 1, EZ-AR™ 2 and AR Ultra Solution in combination with EZ-AR™ Common ensures uniform dewaxing, rehydration and antigen retrieval. The main advantages that these solutions offer are:

1. Eliminates the use of enzymes as pretreatment in most cases, hence removing the guesswork from optimizing treatment time on the basis of tissue fixation.
2. Reduces background staining: EZ-AR™ solutions increase the availability of antigenic epitopes in tissues due to their unique capability of being heated up to a temperature of 107°C. One may need to use Avidin/ Biotin blocks to block endogenous biotin when using Biotin-Streptavidin based detection systems.
3. Reduces incubation time with primary antibodies
4. Better quality staining due to unique properties of solution.
5. Preserves morphology of tissues.
6. Non-toxic and non-flammable.

EZ-AR™ Common, EZ-AR 1, EZ-AR 2 and AR Ultra solutions are recommended for use in the EZ-Retriever® IR System (Cat. No. MW015-IR, MW016-IR). The EZ-Retriever® IR System and accessories offer a time and temperature controlled environment for heating of solutions. Use of any other microwave will result in uncontrolled heating of the solutions thus optimized staining results in any other microwave other than EZ-Retriever® IR System cannot be guaranteed. Please call BioGenex at Toll Free number (800) 421-4149 (USA only) or your local distributor for more information on the above-recommended products and their accessories.

PRODUCT AVAILABILITY

Name	Catalog Number	Format
EZ-AR™ 1 RTU	HK521-XAK	1L
EZ-AR™ 1 RTU	HK521-XIK	2 Gallons
EZ-AR™ 2 RTU	HK522-YRK	500 mL
EZ-AR™ 2 RTU	HK522-XAK	1L
EZ-AR™ 2 RTU	HK522-XIK	2 Gallons
AR Ultra Solution	HK523-YAK	100 mL
AR Ultra Solution	HK523-YRK	500 mL
AR Ultra Solution	HK523-XAK	1L
EZ-AR™ Common, Conc. (5X)	HK545-XOK	1L

PREPARATION OF REAGENTS (This section is applicable only for EZ-AR™ Common, which come in concentrated format)

EZ-AR™ Common

The concentrated format of EZ-AR™ Common is 5X concentrated, and should be diluted five-fold with deionized water (mix one part of EZ-AR™ Common with four parts of deionized water). Shake to mix thoroughly.

METHOD OF USE

BioGenex has optimized the Antigen Retrieval protocol for a number of our antibodies. Please refer to individual antibody datasheets for the recommended Antigen Retrieval protocol.

DeWaxing and Rehydration (EZ-AR™ Common):

1. Place the slides in a tank containing EZ-AR™ Common solution. (Recommended volume for slide tanks is 250 ml ± 5 ml.) If no. of slides are less than 24, then volume will be higher (270-280ml) than the recommended one.
2. Place the slide tank in the EZ-Retriever® IR System and heat the slides for 10 minutes at 70ºC.
3. If antigen retrieval is required, proceed to the Antigen Retrieval step. Otherwise, remove the tank from the EZ-Retriever® IR System and allow the slides to cool for 20mins on the bench.
4. Wash slides in DI water for 5 min. Then transfer them to IHC wash buffer and keep the slides in buffer for 5 min or until they are ready to be stained.

Antigen Retrieval (EZ-AR™ 1, EZ-AR™ 2 and AR Ultra Solution):

1. After the dewaxing step, immediately transfer the slides to a tank containing EZ-AR™ 1or 2 or AR Ultra Solution.
2. Treat for 10 minutes at 107 ºC for EZ-AR™ 1, EZ-AR™ 2 and AR Ultra Solution.
3. Remove the tank from the microwave oven or EZ-Retriever® IR System and allow the slides to cool for 20mins on the bench.
4. Wash slides in DI water for 5 min. Then transfer them to wash buffer used in routine Histology and Special Stains Techniques and keep the slides in buffer for 5 min or until they are ready to be stained.

Note: Please do not use polypropylene Coplin jars as they may melt at high temperature. Use fresh solution for each batch of slides.

STORAGE AND HANDLING

EZ-AR™ Common, EZ-AR™ 1, EZ-AR™ 2 and AR Ultra Solution	Store solution at 2°-8°C

PRECAUTIONS

Solutions will reach high temperature during use, please handle with care. Wear suitable protective clothing, thermal protective gloves and eye/face protection. Not regarded as a health or safety hazard under current legislation.

The EZ-AR™ Common, EZ-AR™ 1, EZ-AR™ 2 and AR Ultra solutions may be discarded after use as any other non-hazardous waste. However, EZ-AR™ Common may not be disposed off in the sink after usage in the dewaxing step. The residual wax in the solution may cause plumbing problems.

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. Some tissues may show heat artifact.

REFERENCE ARTICLES

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 39:741-748, 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.*

The above products and their methods are covered by one or a combination of any of the following patents: U.S. Patent No. 5,244,787; U.S. Patent No. 5,578,452; U.S. Patent No. 6,451,551; U.S. Patent No.

6,632,598; and their foreign equivalents, i.e. European Patent No. 0607422 and Japanese Patent No. 3,108,099; as well as related U.S. and Foreign Patents Pending.