Carezyme III: Pronase Kit

Pretreatment Reagent
901-PRT957-081017

BIOCARE MEDICAL

Catalog Number: **PRT957 KH**

Description: 25 ml, ready-to-use

Intended Use:

For In Vitro Diagnostic Use

Carezyme III: Pronase Kit is a concentrated solution of pronase enzyme and accompanying buffer intended for use as a pretreatment step on formalin-fixed, paraffin-embedded (FFPE) tissues in immunohistochemistry (IHC) and *in situ* hybridization (ISH) procedures. The clinical interpretation of any staining or its absence should be complemented by morphological studies and proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

Pronase (*Streptomyces griseus*) is a commonly used digestive enzyme. In formalinfixed paraffin-embedded tissues, certain antibody or *in situ* hybridization probes require enzyme pretreatment for proper immunohistochemical or *in situ* hybridization staining. CAREZYME III is an aggressive enzyme and can be used at room temperature or at 37ºC. Certain antibodies such as EGFR may require this type of aggressive enzyme digestion. This product is provided as a two component system and can be used at different concentrations. CAREZYME III is not compatible with antibodies AE1/AE3 or AE3.

Known Applications:

Immunohistochemistry and *in situ* hybridization (formalin-fixed paraffin-embedded tissues)

Supplied As:

25 ml Kit

1. Pronase (concentrate) 6 ml (PRT957G)
2. Buffer 25 ml (PRB957H)

Materials and Reagents (Needed But Not Provided):

Microscope slides, positively charged
Desert Chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be replaced with a Xylene substitute*)
Ethanol or reagent alcohol
Decloaking Chamber* (Pressure cooker)
Deionized or distilled water
Wash buffer*(TBS/PBS)
Pretreatment reagents*
Avidin-Biotin Blocking Kit*(Labeled streptavidin kits only)
Peroxidase block*
Protein block*
Primary antibody*
in situ hybridization (ISH) probes*
Negative control reagents*
Detection kits*
Detection components*
Chromogens*
Hematoxylin*
Bluing reagent*
Mounting medium*

* Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Storage and Stability:

Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

Protocol Recommendations:

Deparaffinize tissues and hydrate to water. If necessary perform a hydrogen peroxide block, wash in water, and rinse in PBS or TBS. The tissue section is ready for protease digestion.

Manual IHC:

1. Combine 1 part Pronase concentrate with 4 parts buffer (0.1%). Incubate for 5-10 minutes at 37ºC. (Strong)
2. Combine 1 part Pronase concentrate with 9 parts buffer (0.05%). Incubate for 5-10 minutes at 37ºC. (Medium)

Automated IHC:

1. Combine 1 part Pronase concentrate with 4 parts buffer (0.1%). Incubate for 10-15 minutes at room temperature.

***In situ* Hybridization:**

1. Combine 1 part Pronase concentrate with 9 parts buffer (0.05%). Incubate at room temperature for 10 minutes. It may be necessary to adjust ratio and time for variably fixed tissue.

Protocol Notes:

1. Color-coded buffer (rose) for easy identification.
2. Buffer incorporates ASSURE™ Technology, a color-coded pH indicator. The enduser can visually inspect the solution and see that the mixture or buffer is at the proper pH. If the mixed solution is purple, the pH is too high for optimal digestion. If the buffer or Pronase solution turns orange or yellow, the pH is too low for optimal digestion.
3. The working solution is stable for 7 days at 4ºC.

Limitations:

The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA, USA (www.clsi.org). 2011

Precautions:

1. This product contains less than 0.1% sodium azide. Concentrations less than 0.1%

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

Rev: 062117

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control,1976, National Institute of Occupational Safety and Health, 1976) (1)

2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (2)

3. Microbial contamination of reagents may result in an increase in nonspecific staining.

4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.

5. Do not use reagent after the expiration date printed on the vial.

6. The SDS is available upon request and is located at http://biocare.net.

7. Consult OSHA, federal, state or local regulations for disposal of any toxic substances.

References:

1. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."

2. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

Rev: 062117

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080