

## DATA SHEET

### Protease XXIV Kit for Xmatrix Cat. Nos. EK002-10XE

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Doc. No. 932-EK002-10XE Rev. C

Effective Date: 04-Apr-2022

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#### REAGENTS SUPPLIED

Store at 2-8°C

Protease XXIV (HK053-5K)

4 vials of Liquid Protease

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#### Intended Use

Enzyme digestion kits are intended for in vitro diagnostic use for enzymatic pretreatment of formalin-fixed tissue sections prior to immunohistochemistry (IHC) or in situ hybridization (ISH).

#### Summary and Explanation

The staining pattern and intensity of signal for some IHC and ISH procedures can be improved by using an enzymatic pretreatment of the specimen. Digestive enzyme pre-treatment is recommended prior to the addition of the primary antibody or probe. Pre-treatment conditions are often tissue-and fixation-dependent; they should be tested thoroughly and validated in the user's laboratory. Always consult the protocol supplied with each primary antibody or probe for optimal digestion conditions.

#### Principles of the Procedure

There are a variety of proteolytic enzymes used for pretreatment of tissue sections in IHC suggesting that there is no specific mechanism of action. Enzyme pretreatment for antigen retrieval is effective only for a few antigens. Concentration of enzyme, incubation time and temperature are critical to get the optimal signal to noise ratios in IHC. In ISH, enzyme pretreatment increases the access of probe to target DNA or RNA sequences.

#### Method of Use

1. Remove one vial of Liquid protease from refrigerator and allow it to reach to room temperature.
2. Use 100-200ul of protease according to the size of tissue. After using the vial keep it back to refrigerator.

#### Protocol

Certain antibodies require pretreatment with enzymatic reagents on Xmatrix System. Use digestive enzyme pretreatment as per the protocol given in the system. Always follow the protocol supplied with each primary antibody for optimal pretreatment protocol conditions. Pretreatment conditions may vary for formalin-fixed sections, as results are often tissue and fixation-dependent. Pretreatment conditions should be tested extensively and validated in the user's laboratory.

#### Storage and Handling

Store all reagents at 2-8°C. Do not use after expiration dates as indicated on the reagent labels.

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**For In-Vitro Diagnostic Use.**

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#### Specimen Preparation

Paraffin embedded and formalin fixed tissue sections 4-6 micron thick are suitable for use with enzyme pretreatment.

#### BioGenex Automated Staining System Protocol

For routine immunohistochemistry, use digestive enzyme pretreatment prior to the addition of the primary antibody. Add enough working enzyme solution to entirely cover the tissue section (100 µl) and incubate at 37°C for 10-20 minutes for Protease XXIV. Rinse well with PBS before applying primary antibody. Always consult the protocol supplied with each primary antibody for optimal digestion conditions. Pretreatment conditions may vary for formalin-fixed sections, as results are often tissue- and fixation-dependent. Pretreatment conditions should be tested extensively and validated in the user's laboratory.

\* For exceptions to this pretreatment protocol, refer to the BioGenex antibody datasheet.

#### Quality Control

The antibody datasheet describes the appropriate enzyme to be used. Refer to the antibody package insert for positive control tissues.

#### Trouble shooting

Over digestion or incomplete digestion may result in damage of tissue. Optimize the concentration of enzyme, incubation time and temperature to obtain desired results.

#### Expected Results

Enhanced signal is seen with recommended antibodies after enzyme pretreatment.

#### Limitations of the procedure

All antigens do not need enzyme pretreatment. Immunohistochemistry (IHC) is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can also cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue may cause variations in results (Nadji and Morales, 1983).

#### Performance Characteristics

BioGenex has conducted studies to evaluate the performance of all its pre-treatment enzymes using several BioGenex antibodies and detection systems. BioGenex enzyme kits have shown reproducible and consistent results when used within a single run, between runs, between lots and wherever applicable between manual and automated runs. The products have been determined to be stable for the periods specified on the labels either by standard real time or accelerated testing methods. BioGenex ensures product quality through 100% quality control for all products released and through surveillance programs.

#### Precaution

It contains sodium azide at concentrations of less than 0.1%. Sodium azide is not classified as a hazardous chemical at the concentration of this product. However, toxicity information regarding sodium azide at the product's concentration has not been thoroughly investigated.

**Caution:** The packaging of this product contains natural rubber latex which may cause allergic reactions.

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