In Situ Hybridization and Detection Systems Accessories Proteinase K

Cat. No. 33801

For Research Use Only

INTRODUCTION

Nucleic acid probes labeled with biotin- or fluorescein-modified nucleotides are particularly useful for *in situ* hybridization and detection applications. The ENZO **Proteinase K** provides a very high quality proteinase for pretreatment of formalin-fixed tissue and paraformaldehyde-fixed cells.

REAGENTS

Proteinase K, 2 X 5 mg
Lyophilized powder

STORAGE

Store refrigerated at 2-8°C. The Proteinase K powder is stable until the expiration date on the label.

WARNINGS

- **For RESEARCH use only.**
- Use a safety pipetting device for all pipeting. Never pipet by mouth.

RECOMMENDED PROCEDURES
for use of ENZO Proteinase K

ENZO **Proteinase K** is specifically designed for use in the pretreatment of formalin-fixed tissue and paraformaldehyde-fixed cells in preparation for *in situ* hybridization and detection studies. A brief outline of use of the Proteinase K product follows.

Preparation of Cell and Tissue Specimen Slides

Choose a reliable method for preservation and fixation of the cells and/or tissue to be analyzed. Paraffin-embedded fixed sections must be deparaffinized by soaking in Xylenes and then rehydrated through graded alcohols. Following these procedures, slides of formalin-fixed, paraffin-embedded sections are ready for Proteinase Pretreatment.

Proteinase Pretreatment of Tissues and Cells

Introduction

Formalin-fixed tissue and cells fixed with crosslinking fixatives need to be made permeable to the reagents used for *in situ* hybridization and detection. Thus, both formalin-fixed tissue and paraformaldehyde-fixed cells should be proteinase treated prior to hybridization and detection.

Stock Solution

Prepare a 2.5 mg/ml concentrated stock solution of Proteinase K by dissolving one 5 mg vial of Proteinase K powder in 2 ml of phosphate (or Tris) buffered saline (ENZO Cat. Nos. 33802 or 33803, respectively). Store convenient portions of the solution frozen at ≤-20°C. Freeze all tubes that are not required for immediate use. Once frozen, the enzyme is stable for at least one year. Once defrosted, do not refreeze.

Working Solutions

As needed, use the vials of Proteinase K Stock Solution to prepare working strength solutions of Proteinase K. Initial experiments with formalin-fixed tissue should be performed with 10-fold dilutions of the 2.5 mg/ml Stock Solution. More dilute preparations of Working Solutions may be required to preserve morphology. Once an appropriate dilution of the Proteinase K has been determined for the types of tissue being analyzed, routine experiments can be performed at the pre-determined concentration. Do not store diluted Proteinase K solutions, the working solutions should be used immediately and unused material should be discarded.

Paraformaldehyde-fixed cells will require more dilute preparations of Proteinase K than formalin-fixed tissue. Initial experiments might be performed with 20X to 50X dilutions of the 2.5 mg/ml Stock Solution.

Pretreatment Procedure

Following deparaffinization and rehydration of paraffin-embedded specimens (see above) and following drying of paraformaldehyde fixed cellular slides, **pipet 0.5 ml of freshly prepared Proteinase K Working Solution onto each specimen slide. Incubate the slides at 37°C for 15 minutes.**

Tap off Proteinase K and soak slides at room temperature for 1 minute in a Coplin jar filled with buffered saline wash buffer. Gently agitate the Coplin jar while soaking the slides.

NOTE: *Shorter or longer incubation times can be used. However, to optimize signal response, titration of the Proteinase K is necessary for each new tissue type.*

Tap off wash buffer and proceed to Quench Treatment and/or Dehydration Procedures.

Quench Treatment

When using a horseradish peroxidase-based detection reagent, it is often necessary to quench the activity of endogenous peroxidases. **Apply 0.5 ml of phosphate buffered saline containing 3% H_2O_2 to each specimen slide. Incubate at 37°C for 10-15 minutes**.

Rinse off quench reagent using a stream of wash buffer (phosphate buffered saline) from a squeeze (wash) bottle.

Proceed to the Dehydration Procedure.

Dehydration Procedure

Introduction

Following Proteinase Pretreatment and Quench Treatment, **all slides must be dehydrated prior to beginning the *in situ* hybridization and detection procedures**.

Procedure

Dehydrate slides by incubations at room temperature in the following series of reagents for one minute in each:

Soak Number	*Reagent*	*Duration of Soak*
1	Deionized water	1 minute
2	50% Alcohol	1 minute
3	70% Alcohol	1 minute
4	100% Alcohol	1 minute

Dry the slides at 37°C for 5-10 minutes or at room temperature. The slides can be stored desiccated at 2-8°C for up to one week, if necessary.

Preparation of Probe Reagents

Nick Translated DNA Probes

To prepare probe for *in situ* hybridization, add concentrated DNA probe to give a final concentration of 0.2-5.0 μg/ml in a medium containing 1X to 5X SSC or SSPE, 10% to 50% formamide, and 1% to 10% dextran sulfate. Alternatively, use ENZO ***In Situ*** **Hybridization Buffer**, Cat. No. 33808, for preparation of ready-to-use probe reagents for *in situ* hybridization studies.

Oligonucleotide Probes

To prepare probe for *in situ* hybridization, add concentrated oligonucleotide probe to give a final concentration of 50-100 ng/ml in a medium containing 1X to 5X SSC or SSPE, 10% to 50% formamide, and 1% to 10% dextran sulfate. Alternatively, use ENZO ***In Situ*** **Hybridization Buffer**, Cat. No. 33808, for preparation of ready-to-use oligonucleotide probe reagents for *in situ* hybridization studies.

Hybridization and Post Hybridization Washing

Hybridization

To hybridize to target DNA in fixed, proteinase pretreated tissues and in fixed cells, place a drop of ready-to-use probe reagent on the specimen and cover with a coverslip, being careful not to trap bubbles under the coverslip. Heat the slide on a heating block set to 95°C for 8-10 minutes for tissue specimens and 4-5 minutes for cellular specimens.

Remove slide to a 37°C slide warmer and incubate for 30-60 minutes (up to 24 hours, depending upon the probe concentration and the target nucleic acid level). Note that extended hybridizations are best carried out in an humidified chamber.

Post Hybridization Washing

After hybridization, remove the coverslip from the slide and soak the slide in a buffered saline solution (phosphate buffered saline, ENZO Cat. No. 33802, for horseradish peroxidase developments; Tris buffered saline, ENZO Cat. No. 33803, for alkaline phosphatase developments) for 5 minutes at room temperature.

Remove the slide from the buffer and wipe around the specimen. Pipet 0.5-1.0 ml of a post hybridization wash solution onto the slide and incubate the slide at 37°C for 10-20 minutes. For a nick translated probe, use either ENZO Cat. No. 33809, ***In Situ*** **Hybridization Wash Reagent**, or a solution containing 50% deionized formamide in 0.2X to 1X SSC. For oligonucleotide probes, use 37°C pre-warmed 1X SSC for 10 minutes at 37°C, followed by 37°C pre-warmed 0.2X SSC for 10 minutes at 37°C for post hybridization washes.

Following post hybridization washes, soak the slide twice for 2 minutes in buffered saline.

Detection of *In Situ* Hybridized Probes

Choose a good quality detection system for either colorimetric or fluorescence detection of the hybridized probe.

For Technical Assistance contact Enzo:
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