Product Manual

In Situ Hybridization and Detection Systems Accessories

Wash Buffer Salts

Catalog #: ENZ-33802

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Nucleic acid probes labeled with hapten-modified nucleotides are particularly useful for *in situ* hybridization applications. ENZO ***Wash Buffer Salts*** provides a high quality hybridization wash solution for *in situ* hybridization analyses using DNA probes labeled with biotin-, or fluorescein-modified nucleotides.

REAGENTS

Wash Buffer Salts, 1L

Lyophilized powder containing salts for 1L of phosphate buffered saline.

FOR USE

Dissolve entire contents of packet in 1 liter of distilled or deionized water. The pH of the solution should be 7.1 ± 0.1.

STORAGE

Store lyophilized powder at room temperature (18-28°C). Store reconstituted buffer at 2-8°C.

WARNINGS

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

RECOMMENDED PROCEDURES

for use of ENZO *Wash Buffer Salts*

ENZO ***Wash Buffer Salts***, Cat. No. ENZ-33802, are used in the preparation and pretreatment of tissue and cellular specimen slides and in the post-hybridization steps of *in situ* detection procedures. Use ENZO ***Wash Buffer Salts***, Cat. No. ENZ-33802 (Phosphate buffered saline), when using a peroxidase-based detection reagent. Use ENZO SignaSure® ENZO Cat. No. ENZ-33803 (Tris buffered saline), when using an alkaline phosphatase-based detection reagent.

Preparation of Cell and Tissue Specimen Slides

Choose a reliable method for preservation and fixation of the cells and/or tissue to be analyzed. When using horseradish peroxidase detection systems, inactivate endogenous peroxidases by pretreatment with a Quench Reagent (Phosphate buffered saline containing 3% H_2O_2).

Proteinase Pretreatment of Tissues and Cells

ENZO ***Wash Buffer Salts*** are appropriate for use with Proteinase K, Cat. ENZ-33801, in the pretreatment of formalin-fixed tissue and paraformaldehyde-fixed cells in preparation for *in situ* hybridization analysis. Formalin 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.

Hybridization and Post Hybridization Washing

A brief outline of hybridization, post hybridization washing and detection methods follows.

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 (or 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. ENZ-33802, for horseradish peroxidase developments; Tris buffered saline, ENZO Cat. No. ENZ-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 ENZO *in situ* Hybridization Wash Reagent Cat. ENZ-33809 onto the slide. Incubate the slide at 37°C for 10-20 minutes. 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.

USE FOR RESEARCH PURPOSES ONLY

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TRADEMARKS AND PATENTS

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Please contact Enzo Life Sciences Technical Support if necessary.

GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com