In Situ Hybridization and Detection Systems Accessories

In Situ Hybridization Buffer

Cat. No. 33808

For Research Use Only

INTRODUCTION

Nucleic acid probes labeled with hapten-modified nucleotides are particularly useful for *in situ* hybridization applications. ENZO ***In Situ* Hybridization Buffer** provides a high quality hybridization medium for *in situ* hybridization analyses using either DNA or oligonucleotide probes labeled with biotin- or fluorescein-modified nucleotides.

REAGENTS

***In Situ* Hybridization Buffer**, 10 ml (1.25X concentrate)
Sodium phosphate, NaCl and EDTA (SSPE) buffered formamide, containing hybridization enhancers

STORAGE

1. Store refridgerated at 2-8°C. The *In Situ* Hybridization Buffer is stable for up to 2 years when stored refridgerated in a well sealed bottle.
2. If precipitation (crystallization) is evident, mix the *In Situ* Hybridization Buffer vigorously while warming to 50-60°C.

WARNINGS

- **For RESEARCH use only.**
- Use a safety pipetting device for all pipeting. Never pipet by mouth.
- The *In Situ* Hybridization Buffer contains formamide which is a combustible teratogen. Keep away from open flame. Pregnant workers should keep exposure to a minimum. Avoid inhalation, ingestion and contact with skin. Wash thoroughly with soap and water if contact is made. Dispose of this solution in accordance with local regulations.

RECOMMENDED PROCEDURES

for use of ENZO *In Situ* Hybridization Buffer

ENZO ***In Situ* Hybridization Buffer**, Cat. No. 33808, provides a sufficient amount of hybridization buffer concentrate to prepare 12.5 ml of ready-to-use probe reagent solutions.

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 such as 3% hydrogen peroxide in phosphate-buffered saline.

Preparation of Probe Reagents

To prepare ready-to-use probe reagent using the **ENZO *In Situ* Hybridization Buffer**, follow these instructions:

Nick Translated DNA Probes

For each milliliter of ready-to-use probe reagent, pipet **0.8 ml of *In Situ* Hybridization Buffer** into a clean tube. Add concentrated DNA probe to give a final concentration of 0.2-5.0 μg/ml (do not exceed 0.2 ml of probe). **Bring the volume to 1.0 ml** with distilled or deionized water. Mix vigorously. Store probe reagent at 2-8°C when not in use and warm to room temperature prior to use.

Oligonucleotide Probes

For each milliliter of ready-to-use oligonucleotide probe reagent, pipet **0.8 ml of *In Situ* Hybridization Buffer** into a clean tube. Add concentrated oligonucleotide probe to give a final concentration of 50-100 ng/ml (do not exceed 0.2 ml of probe). **Bring the volume to 1.0 ml** with distilled or deionized water. Mix vigorously. Store probe reagent at 2-8°C when not in use and warm to room temperature prior to use.

Hybridization and Post Hybridization Washing

For complete instructions for *in situ* hybridization and detection, refer to ENZO User's Guide 102 for *in situ* use of nick translated DNA probe or ENZO User's Guide 105 for general instructions for *in situ* hybridizations and detections. 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. 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.

See the ENZO catalog for a complete list of products available for use with ENZO *In Situ* Hybridization Buffer.

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