I. INTRODUCTION

A. TSA and In Situ Hybridization (ISH)

What is ISH?

In situ hybridization (ISH) is a technique to detect, visualize and localize DNA and RNA at the cellular level. Detection schemes for in situ hybridization include autoradiography for radioactive probes, dye deposition for enzyme labeled probes and fluorescence for fluorescent probes. As sophisticated equipment, such as fluorescence microscopes, CCD cameras, and imaging software has evolved, fluorescence detection has become one of the most powerful tools in the research laboratories. FISH (fluorescent in situ hybridization) is used extensively in molecular cytogenetics for chromosome mapping studies and in infectious disease research for virus detection.

What is TSA?

TSA™ (Tyramide Signal Amplfication) is a powerful technology from NEN™ Life Science Products that enhances both chromogenic and fluorescent signals up to 1,000 fold. It is easily integrated into standard IHC or in situ hybridization protocols, provided that Horseradish Peroxidase (HRP) is in the system.

How does Signal Amplification Work?

TSA technology uses HRP to catalyze the deposition of biotin or fluorophore labeled tyramide onto tissue sections or cell preparation surfaces that have been previously blocked with proteins. The reaction is quick (less than 10 minutes) and results in the deposition of numerous biotin or fluorescent labels close to the enzyme (see figure 1). These labels can be directly or indirectly detected by standard techniques, with significant enhancement of the signal. Because the added labels are deposited adjacent to the enzyme site, there is minimal loss in resolution. This signal amplification technique may be applied to both IHC and ISH.

How is TSA used in ISH?

Standard nonradioactive DNA or RNA probe labeling, hybridization and immunological protocols are compatible with TSA. Many mechanisms for probe labeling are available to the user. Enzymatic methods using Fluorescein-N^6-dATP, Fluroescein- N^6-ATP and Fluorescein-N^6-ddATP can be used in nick translation and random priming, in in vitro transcription, and in 3' end labeling respectively. These fluorescent nucleotides and Antifluorescein HRP conjugates are available from NEN™ Life Science Products. Other commercial methods available using chemical addition or haptenization are SulfoPROBE and PhotoBiotin™. In addition, direct horseradish peroxidase (HRP) linking to DNA can be accomplished by a variety of methods.

What is the difference

TSA-Indirect deposits numerous biotinyl tyramides, as described above. These deposited biotins are detected with a conjugated

between TSA-Indirect and TSA-Direct?

streptavidin. If chromogenic visualization is selected, Streptavidin-Horseradish Peroxidase (SA-HRP) or Streptavidin-Alkaline Phosphatase (SA-AP) is followed by the appropriate chromogen. For fluorescent detection with TSA-Indirect, a variety of fluorescent conjugated streptavidins may be used.

TSA-Direct simplifies fluorescent detection. Fluorescent tyramides are deposited directly onto the surface and visualized immediately with fluorescent microscopy. The fluorescent tyramides are available in TSA-Direct include fluorescein (Green), tetramethylrhodamine (Red), coumarin (Blue), and Cyanine 3 (Red) providing researchers with a wide spectrum of options. Applications using IHC, ISH and immunocytochemistry have shown that TSA direct fluorescent detection is often as sensitive as TSA indirect fluorescent detection.

TSA also provides users with the option of sequential multicolor detection. Protocols are available from NEN™ Life Science Products upon request.

What ISH and IHC media are compatible with TSA?

TSA has been successfully applied to the following media: chromosome spreads, cultured cells, cryostat sections, and formalin-fixed/paraffin-embedded sections as well as some plastic embedded sections (e.g. MMA).

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