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**DNA PROBE HYBRIDIZATION/DETECTION SYSTEM IN SITU KIT**  
**A Complete Southern Hybridization & Immunodetection System**  
**Cat No. IH-60033 (SHD-1500): Sensitive 100 Slides**  
**Cat No. IH-60034 (SHD-1502): Ultra-Sensitive 100 Slides**

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**INSTRUCTION  
MANUAL**

ID-M10206  
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## TABLE OF CONTENTS

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<b>I.</b>	<b>Introduction</b>	<b>3</b>
<b>II.</b>	<b>Principle</b>	<b>3</b>
<b>III.</b>	<b>Materials and Reagents supplied</b>	<b>4</b>
<b>IV.</b>	<b>Materials and Reagents needed</b>	<b>4</b>
<b>V.</b>	<b>Procedure</b>	<b>5-6</b>
<b>VI.</b>	<b>Quality Control</b>	<b>7</b>
<b>VII.</b>	<b>Troubleshooting</b>	<b>7</b>
<b>VIII.</b>	<b>Precautions and Storage</b>	<b>8</b>
<b>IX.</b>	<b>References</b>	<b>8-9</b>

## I. INTRODUCTION

The DNA Probe Hybridization/Detection System - Southern Kit is a complete hybridization and immunodetection system, incorporating the biotin-streptavidin amplified technology. Optimized for Southern hybridization, these user-friendly kits provide consistent results and maximum sensitivity to ensure economical and efficient use of the nucleic acid probes. (See Maxim catalog for detailed biotinylated probes)

The DNA Probe Hybridization/Detection System is designed for the specific enzymatic detection of biotinylated DNA probes following hybridization to target DNA sequences transferred onto membrane supports (Southern hybridization).

This complete hybridization/detection kit can be used with a large variety of commercial or user labeled biotinylated probes. With the amplified system, stronger signal strength can be obtained with significantly smaller amounts of DNA. A high stringency Hybridization buffer is included for dilution of DNA probes.

For optimal results, please read and follow the instructions in this manual carefully. If you have any questions, contact Maxim Biotech Customer Service at (415) 871-1919.

This product is intended for research use only and not for diagnostic purposes.

## II. PRINCIPLE

While early detection methods relied on the use of radioactive probes, non-radioactive labeling methods have recently gained in popularity. The most widely used non-radioactive technique entails labeling the probe with biotin. The hybridized probe is then detected by addition of enzyme-conjugated streptavidin followed by a suitable enzyme substrate, which produces a colored end product. This conventional colorimetric reaction avoids the health hazards, disposal problems, and inherent instability of radio-labeled probes.

The DNA Probe Hybridization/Detection System - Southern Kit (High-Sensitivity and Ultra-Sensitivity) was developed at Maxim Biotech to provide the convenience of a colorimetric assay combined with sensitivity approaching that of radioactive methods.

For **High-Sensitivity Kit**: To detect a biotinylated probe hybridized to the target sequence, a streptavidin-alkaline phosphatase conjugate is applied. Upon addition of the component BCIP/NBT substrate, an intense purple signal appears at the specific site of the hybridized probe. This directly streptavidin-alkaline phosphatase conjugate linked to the biotinylated probe provides a rapid and highly sensitive detection method.

For **Ultra-Sensitivity Kit**: A higher sensitivity is obtained which is accomplished by amplification of the initial hybridization reaction using additional binding steps in which biotin is re-introduced into the system. These intermediate steps utilize the high affinity and high fidelity of antibody binding. To detect a biotinylated probe hybridized to the target sequence, an anti-biotin antibody is first applied. This binds to the biotin on the hybridized probe. In order to re-introduce biotin into the system, an immunoglobulin that is coupled with substantially higher numbers of biotin moieties than the probe is then added. Biotinylated anti-mouse Ig recognizes and binds to the anti-biotin antibody. This additional layer initiates amplification of the signal. To visualize the antibody/probe complex, alkaline phosphatase-conjugated streptavidin is added next. Each biotin molecule on biotinylated anti-mouse Ig, as well as any free biotin on the nucleic acid probe, is bound by a streptavidin moiety of the Conjugate. Each molecule of conjugate contains up to six alkaline phosphatase molecules, providing further amplification of the reaction. Upon addition of the BCIP/NBT substrate, an intense purple signal appears at the specific site of the hybridized probe.

### III. SUPPLIED MATERIALS AND REAGENTS

Kit Component	Cap Color	Volume	IH-60033	IH-60034
Hybridization buffer		200 ml	+	+
20X Post-hybridization wash buffer		50 ml	+	+
Blocking powder		25g	+	+
1X AP diluent		250 ml	+	-
2X AP diluent		250 ml	-	+
Mouse anti-biotin antibody	Brown	200 $\mu$ l	-	+
Biotinylated anti-mouse antibody	Yellow	200 $\mu$ l	-	+
Streptavidin-alkaline phosphatase conjugate	Red	400 $\mu$ l	+	+
BCIP/NBT substrate		200 ml	+	+
20X Enhancer wash buffer		100 ml	+	+
Instruction manual			+	+

### IV. NEEDED MATERIALS AND REAGENTS

- Oven(s) or incubator(s), 37°C, 42- 45°C
- Pipettes
- Pipette tips
- Your biotinylated DNA probes
- Forceps
- TBS
- Timer
- Heating block or oven 95°C
- Distilled or deionized H<sub>2</sub>O

## V. PROCEDURE

### A. PREPARATION OF REAGENTS:

- Post-hybridization wash buffer: Dilute 50 ml of 20X concentrated Post-Hybridization wash buffer into 950 ml ddH<sub>2</sub>O. Store at room temperature (\*Contain SDS, warm up before dilution).
- Blocking solution: Dissolve the powder with 500 ml TBS. Store at 4 °C.
- 1X AP diluent: Dilute 250 ml 2X AP diluent with 250 ml ddH<sub>2</sub>O. Store at 4 °C.
- Enhancer wash buffer: Dilute 100 ml of 20X concentrated Enhancer wash buffer into 1900 ml ddH<sub>2</sub>O. Stored at room temperature.
- Mouse anti-biotin antibody solution: Dilute 1:1,000 with 1X AP diluent before use. Store at 4 °C. (Only available in Ultra-Sensitivity Kits)
- Biotinylated anti-mouse antibody solution: Dilute 1:1,000 with 1X AP diluent before use. Store at 4 °C. (Only available in Ultra-Sensitivity Kits)
- Streptavidin-alkaline phosphatase conjugate: Make 1:500 dilution with 1X AP diluent before use. Store at 4 °C.

### B. DILUTION OF PROBES

The DNA Probe Hybridization/Detection System - Southern Kit may be used to detect any properly biotinylated probe. Due to the high sensitivity of this kit, it is recommended that DNA probes be diluted to 100-200 ng/ml prior to use. Commercially available probes should be diluted approximately 5- to 10-fold. Hybridization buffer is provided for dilution of commercial probes. To obtain working DNA probe/Hybridization buffer, perform dilutions in autoclaved tubes as follows:

- 5-fold dilution: add 1 part probe to 4 parts Hybridization buffer
- 10-fold dilution: add 1 part probe to 9 parts Hybridization buffer

**CAUTION:** Use of commercial probes at full strength may result in non-specific binding and low signal to noise ratio. If you would like information about our specific probes, call Maxim Customer Service at (415) 871-1919.

### C. HYBRIDIZATION AND DETECTION

**NOTE:** The membranes should not be allowed to dry out at any time during the procedures. All reagents used during hybridization and detection should be warmed to room temperature before use.

1. Place the working DNA probes in an oven or heating block at 95 °C for 5-10 minutes to denature the DNA. (Not necessary for Oligo-Probe).
2. Ice quench the denatured DNA probes.
3. Mix thorough the denatured DNAs with Hybridization buffer at 10-20 ng/ml final probe concentrations.
4. Add the probe solutions to target transferred membrane and incubate at 37-45 °C for 2 hours, or longer if desired.

## V. PROCEDURE Continued

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5. Wash membrane with Post-hybridization wash buffer at 37 °C for 10 minutes, 2X.
6. Incubate membrane with the Blocking solution at 37 °C for 30-60 minutes with mild agitation.
7. Incubate membrane with the Mouse anti-biotin Ab at 37 °C for 60 minutes, mild agitation. (Skip for High Sensitivity)
8. Wash membrane with the Enhancer wash buffer 5 minutes, 3X. (Skip for High-Sensitivity Kit)
9. Incubate membrane with the Biotinylated anti-mouse Ab at 37 °C for 30 minutes, mild agitation. (Skip for High-Sensitivity Kit)
10. Wash membrane with the Enhancer wash buffer 5 minutes, 3X. (Skip for High-Sensitivity Kit)
11. Incubate membrane with the Streptavidin-alkaline phosphatase conjugate at 37 °C for 20 minutes, mild agitation.
12. Wash membrane with the Enhancer wash buffer 5 minutes, 3X.
13. Add BCIP/NBT substrate at room temperature until the best signal to noise ratio. Stop with excess ddH<sub>2</sub>O.

## VI. QUALITY CONTROL

Each Southern hybridization assay should include control DNAs to confirm that

1. the detection system is working properly
2. the positive or negative staining is specific
3. the correct procedure has been followed.

**Positive control DNA:** The positive DNA should be prepared from tissue or cells that are known to contain the target nucleic acid. (See Maxim Catalog - Molecular Biology: Positive Control DNA Section)

**Positive control probe:** The biotinylated control DNA probe provided is an human Alu specific oligo-DNA probe. Ideally, the control probe should recognize all DNAs of human origin. (See Maxim Catalog - Molecular Biology: Stock-Oligo Section, Biotinylated Oligonucleotide Probes are available for human, bacteria, parasites and viruses)

## VII. TROUBLESHOOTING

Proper use of this hybridization/detection system will result in an intense blue-black stain at the specific site of the hybridized probe in positive test tissue and positive controls. If staining is absent from any positive control slides, or present in any negative control slides, the test should be considered invalid. If deviation from the expected results occurs, please consult the following trouble shooting guide for assistance. The interpretation of any test result is solely the responsibility of the user.

Observation:	Possible Cause:
A. Sections fall off slides:	<ol style="list-style-type: none"><li>1. Sections not floated in protein-free water bath. Gelatin interfering with adhesion.</li><li>2. Adhesive absent or insufficient.</li><li>3. Insufficient baking of tissue to slides.</li><li>4. Over-digestion with Proteinase K. Try decreasing digestion time or lowering concentration of enzyme.</li><li>5. Denaturing temperature too high or time of denaturing too long.</li></ol>
B. High background staining:	<ol style="list-style-type: none"><li>1. Probe was too concentrated, causing spillover of signal.</li><li>2. Slides dried out during incubations.</li><li>3. Washing steps omitted or too short.</li><li>4. Detergent wash buffer not used after Conjugate incubation.</li></ol>
C. Weak Staining:	<ol style="list-style-type: none"><li>1. Reagents not warmed to room temperature, or room temperature too low. Try longer incubations.</li><li>2. Probe or target DNA not sufficiently denatured. Check heating block temperature. Or try increasing time of denaturation.</li><li>3. Hybridization incomplete. Try increasing time of hybridization.</li></ol>

Correct treatment of tissues prior to and during fixation, embedding, and sectioning is important for obtaining optimal results. Inconsistent results may be due to variations in tissue processing, as well as inherent variations in tissue. The results from in situ hybridization must be correlated with other laboratory findings. If you have questions regarding either the use of the reagents in this kit or the results obtained, contact Maxim Customer Service.

## VIII. PRECAUTIONS AND STORAGE

### Precautions

Precautions: The Blocking solution, AP diluent, Mouse anti-biotin antibody, Biotinylated anti-mouse antibody, and Streptavidin-alkaline phosphatase conjugate reagents in this kit contain sodium azide. The National Institute of Occupational Safety and Health (NIOSH) has issued a bulletin citing the potential explosion hazard due to the reaction of sodium azide with copper, lead, brass, or solder in plumbing systems. Although sodium azide is added at minimal concentration, it is recommended that a copious amount of water be used to flush the drain pipeline after disposal of these reagents in the plumbing system.

The Hybridization Solution reagent in this kit contains formamide. Formamide is classified as a teratogen and pregnant workers should keep exposure to a minimum. Avoid inhalation, ingestion, and contact with unprotected skin. If skin contact occurs, wash thoroughly with soap and water.

### Storage

The reagents in this kit are to be stored at 2-8°C. Reconstituted Proteinase K (10X concentrated solution) should be stored frozen in small aliquots at -20°C.

### Expiration

When stored under the recommended conditions, the performance of the reagents in this kit is guaranteed for 12 months.

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