SUPPLEMENTAL PROTOCOL

In-Situ Hybridization Detection

vector LABORATORIES
Together we breakthrough™

Vector Laboratories offers a wide range of detection methods that encompass visualization preferences and sensitivity requirements when performing in-situ hybridization. Outlined below are examples of the reagents that can be used to detect biotin and fluorescein (the most common labels) using fluorescent or chromogenic techniques. Detection of other labels (Dinitrophenyl, Texas Red™, Rhodamine, Coumarin) can be achieved by substituting the appropriate antibody listed in our catalog or on our website.

Fluorescent In-Situ Hybridization (FISH)

For detection of biotinylated probes, use the following reagents:

- ✓ Fluorescein Avidin DCS
- ✓ Biotinylated Anti-Avidin D
- ✓ VECTASHIELD® Mounting Medium *(with or without counterstain)*

For detection of fluorescein-labeled probes, use the following reagents:

- ✓ Biotinylated Anti-Fluorescein
- ✓ Fluorescein Avidin DCS
- ✓ VECTASHIELD® Mounting Medium *(with or without counterstain)*

Chromogenic In-Situ Hybridization

For detection of biotinylated probes, use the following reagents:

- ✓ Alkaline Phosphatase Streptavidin
- ✓ BCIP/NBT Substrate Kit
- ✓ Levamisole Solution
- ✓ VectaMount® Mounting Medium

For detection of fluorescein-labeled probes, use the following reagents:

- ✓ Alkaline Phosphatase Anti-Fluorescein
- ✓ BCIP/NBT Substrate Kit
- ✓ Levamisole Solution
- ✓ VectaMount® Mounting Medium

Protocols for in-situ hybridization detection are outlined in the following pages. For additional guidelines on the enzymatic or fluorescent detection of ISH probes, please see our website for a comprehensive listing of detection reagents.

Detection Reagents	Cat. No	Amount
Streptavidin, Alkaline Phosphatase	SA-5100	1 ml
BCIP/NBT Substrate Kit	SK-5400	1 kit
Biotinylated Anti-Avidin D	BA-0300	0.5 mg
Biotinylated Anti-Fluorescein	BA-0601	0.5 mg
Fluorescein Avidin DCS	A-2011	1.0 mg
Levamisole Solution	SP-5000	18 ml

Mounting Media	Cat. No	Amount
VECTASHIELD Vibrance® Antifade Mounting Medium	H-1700	10 ml
VECTASHIELD Vibrance® Antifade Mounting Medium with DAPI	H-1800	10 ml
VECTASHIELD® PLUS Antifade Mounting Medium	H-1900	10 ml
VECTASHIELD® PLUS Antifade Mounting Medium with DAPI	H-2000	10 ml

Continued on next page.

6737 Mowry Avenue, Newark, CA 94560
vectorlabs.com

LIT3061.LBL-02558.REV00

Continued from page 1

Fluorescent Detection of Biotin-Labeled ISH Probes

This procedure uses successive rounds of Fluorescein Avidin DCS and Biotinylated Anti-Avidin to detect and amplify in-situ hybridization signals. The multiple binding capacities of Biotinylated Anti-Avidin provide the potential for significant amplification. This antibody binds to Avidin through the antigen binding sites or through the biotin residues that are covalently attached to the molecule. Following the first application of Fluorescein Avidin DCS, the signal is amplified by incubation with Biotinylated Anti-Avidin, followed by a second incubation with Fluorescein Avidin DCS. This procedure results in the introduction of several more fluorochromes at the target site.

1. After hybridization of labeled DNA/RNA probes, block tissue sections or chromosome spreads for ≥ 30 min in a blocking solution such as Casein Solution (Cat. No. SP-5020-250) or Bovine Serum Albumin, Immunohistochemical Grade (Cat. No. SP-5050-500). The effectiveness of the blocking solution may be enhanced by pre-warming the solution to 37°C and incubating tissue sections/ chromosome spreads for 30 min or longer at 37°C.

   Note: 5% nonfat dry milk plus 0.1% Tween® 20 in 4x SSC (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.) can be used as an alternative blocking solution. However, non-fat dry milk can contain variable amounts of biotin which could reduce staining if used as a diluent for (strept)avidin conjugates.

2. Dilute each of the detection reagents, Fluorescein Avidin DCS (Cat.No. A-2011) and Biotinylated Anti-Avidin (Cat.No. BA-0300), to 5 µg/ml approximately 30 min before use to minimize non-specific binding.

   Note: This procedure will require twice the volume of Fluorescein Avidin DCS as Biotinylated Anti-Avidin.

3. Tip off the blocking solution and add the Fluorescein Avidin DCS solution (5 µg/ml). Incubate for 30 min at room temperature.
4. Wash slide for 2 x 3 min.

If satisfactory sensitivity has been achieved, skip to step 8. For increased sensitivity, continue with steps 5 through 7.

5. Incubate with the Biotinylated Anti-Avidin solution (5 µg/ml) for 30 min at room temperature.
6. Wash slides for 2 x 3 min.
7. Follow with a second incubation of the same Fluorescein Avidin DCS solution (5 µg/ml) for 30 min at room temperature.
8. Wash slides 2 x 5 min in 4x SSC + 0.1% Tween 20 before coverslipping with any one of the following mounting media: VECTASHIELD Vibrance® (Cat. No. H-1700), VECTASHIELD Vibrance® with DAPI (Cat. No. H-1800), VECTASHIELD® PLUS (Cat. No. H-1900), and VECTASHIELD® PLUS with DAPI (Cat. No. H-2000).

Fluorescent Detection of Fluorescein-Labeled ISH Probes

1. After hybridization of labeled DNA/RNA probes, block tissue sections or chromosome spreads for ≥ 30 min in a blocking solution such as Casein Solution (Cat. No. SP-5020-250) or Bovine Serum Albumin, Immunohistochemical Grade (Cat. No. SP-5050-500). The effectiveness of the blocking solution may be enhanced by pre-warming the solution to 37°C and incubating tissue sections/ chromosome spreads for 30 min or longer at 37°C.

   Note: 5% nonfat dry milk plus 0.1% Tween 20 in 4x SSC (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.) can be used as an alternative blocking solution. However, non-fat dry milk can contain variable amounts of biotin which could reduce staining if used as a diluent for (strept)avidin conjugates.

2. Dilute each of the detection reagents, Biotinylated Anti-Fluorescein, (Cat. No. BA-0601) and Fluorescein Avidin DCS (Cat.No. A-2011), to 10 µg/ml for approximately 30 min before use to minimize non-specific binding.

Continued on next page.

6737 Mowry Avenue, Newark, CA 94560
vectorlabs.com

LIT3061.LBL-02558.REV00

Continued from page 2

Fluorescent Detection of Fluorescein-Labeled ISH Probes *(cont.)*

3. Tip off the blocking solution and incubate with Biotinylated Anti-Fluorescein solution (10 µg/ml) for 30 min at room temperature.
4. Wash slides for 2 x 3 min.
5. Incubate with the Fluorescein Avidin DCS solution (10 µg/ml) for 30 min at room temperature.
6. Wash slides 2 x 5 min in 4x SSC + 0.1% Tween 20 before coverslipping with any one of the following mounting media: VECTASHIELD Vibrance® (Cat. No. H-1700), VECTASHIELD Vibrance® with DAPI (Cat. No. H-1800), VECTASHIELD® PLUS (Cat. No. H-1900), and VECTASHIELD® PLUS with DAPI (Cat. No. H-2000).

Chromogenic Detection of Biotin-Labeled ISH probes

1. After hybridization of labeled DNA/RNA probes, block tissue sections or chromosome spreads for ≥ 30 min in a blocking solution such as Casein Solution (Cat. No. SP-5020-250) or Bovine Serum Albumin, Immunohistochemical Grade (Cat. No. SP-5050-500). The effectiveness of the blocking solution may be enhanced by pre-warming the solution to 37°C and incubating tissue sections/chromosome spreads for 30 min or longer at 37°C.

   Note: 5% nonfat dry milk plus 0.1% Tween® 20 in 4x SSC (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.) can be used as an alternative blocking solution. However, non-fat dry milk can contain variable amounts of biotin which could reduce staining if used as a diluent for (strept)avidin conjugates.

2. Dilute Streptavidin, Alkaline Phosphatase (Cat.No. SA-5100) 1:200–1:1000 approximately 30 min before use to minimize non-specific binding.
3. Tip off the blocking solution and incubate with diluted Streptavidin, Alkaline Phosphatase solution for 30 min at room temperature.
4. Wash slide for 2 x 3 min in 100 mM Tris, pH 9.5 buffer.
5. Visualize the stain by incubating the tissue section or chromosome spread in BCIP/NBT substrate working solution prepared according to kit instructions (BCIP/NBT Substrate Kit, Cat. No. SK-5400). Incubate until desired sensitivity is achieved.

   Note: For an overnight incubation in the BCIP/NBT substrate solution, use the Streptavidin, Alkaline Phosphatase reagent at a dilution of approximately 1:2500.

6. Wash in 100 mM Tris, pH 9.5 buffer for 5 min.
7. Rinse in tap water and counterstain if desired. BCIP/NBT substrate is compatible with Vector® Nuclear Fast Red counterstain, Cat.No. H-3403, and Vector® Methyl Green, Cat.No. H-3402).
8. For permanent mounting, dehydrate, clear, and mount sections in VectaMount® Mounting Medium (Cat. No. H-5000) which minimizes crystal formation in mounted sections. For aqueous mounting, use VectaMount® AQ Mounting Medium (Cat. No. H-5501).

Continued on next page.

6737 Mowry Avenue, Newark, CA 94560
vectorlabs.com

LIT3061.LBL-02558.REV00

Continued from page 3

Chromogenic Detection of Fluorescein-Labeled ISH Probes

1. After hybridization of labeled DNA/RNA probes, block tissue sections or chromosome spreads for ≥ 30 min in a blocking solution such as Casein Solution (Cat. No. SP-5020-250) or Bovine Serum Albumin, Immunohistochemical Grade (Cat. No. SP-5050-500). The effectiveness of the blocking solution may be enhanced by pre-warming the solution to 37°C and incubating tissue sections/ chromosome spreads for 30 min or longer at 37°C.

   Note: 5% nonfat dry milk plus 0.1% Tween 20 in 4x SSC (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.) can be used as an alternative blocking solution. However, non-fat dry milk can contain variable amounts of biotin which could reduce staining if used as a diluent for (strept)avidin conjugates.

2. Tip off the blocking solution and incubate with alkaline phosphatase anti-fluorescein working solution for 30 min at room temperature.
3. Wash slide for 2 x 3 min in 100 mM Tris, pH 9.5 buffer.
4. Visualize the stain by incubating the tissue section or chromosome spread in BCIP/NBT substrate working solution prepared according to kit instructions (BCIP/NBT Substrate Kit, Cat. No. SK-5400). Incubate until desired sensitivity is achieved.

   Note: For an overnight incubation in the BCIP/NBT substrate solution, use the alkaline phosphatase anti-fluorescein reagent at a concentration of 0.2–2.0 µg/ml.

5. Wash in 100 mM Tris, pH 9.5 buffer for 5 min.
6. Rinse in tap water and counterstain if desired. BCIP/NBT substrate is compatible with Vector® Nuclear Fast Red counterstain, Cat. No. H-3403, and Vector® Methyl Green, Cat. No. H-3402.
7. For permanent mounting, dehydrate, clear, and mount sections in VectaMount® Mounting Medium (Cat.No. H-5000), which minimizes crystal formation in mounted sections. For aqueous mounting, use VectaMount® AQ Mounting Medium (Cat.No. H-5501).

6737 Mowry Avenue, Newark, CA 94560
vectorlabs.com

LIT3061.LBL-02558.REV00