In Situ Hybridization Detection

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:

- 5x ISH Blocking Solution
- Fluorescein Avidin DCS
- Biotinylated Anti-Avidin D
- Vectashield® Mounting Medium (with or without counterstain)

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

- 5x ISH Blocking Solution
- Biotinylated Anti-Fluorescein
- Fluorescein Avidin DCS
- Vectashield® Mounting Medium (with or without counterstain)

Chromogenic *In Situ* Hybridization

For the detection of biotinylated probes, use the following reagents:

- 5x ISH Blocking Solution
- Alkaline Phosphatase Streptavidin
- BCIP/NBT Substrate Kit
- Levamisole Solution
- VectaMount™ Mounting Medium

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

- 5x ISH Blocking Solution
- 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 request the "*In Situ* Hybridization Detection Systems" brochure or visit our website. Please see our catalog or website for a comprehensive listing of detection reagents.

Detection Reagents

5x ISH Blocking Solution	MB-1220	•	100 ml
Alkaline Phosphatase Streptavidin	SA-5100	•	1 ml
Alkaline Phosphatase Anti-Fluorescein	MB-2100	•	150 µg
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

Vectashield® Mounting Medium	H-1000	•	10 ml
Vectashield® Mounting Medium with DAPI	H-1200	•	10 ml
Vectashield® Mounting Medium with PI	H-1300	•	10 ml
Vectashield® Hard•Set™ Mounting Medium	H-1400	•	10 ml
Vectashield® Hard•Set™ Mounting Medium with DAPI	H-1500	•	10 ml
VectaMount® Permanent Mounting Medium	H-5000	•	60 ml
VectaMount™ AQ Aqueous Mounting Medium	H-5501	•	60 ml

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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 with biotinylated DNA/RNA probes, block tissue sections or chromosome spreads for ≥ 30 minutes in 1x ISH Blocking Solution (5x ISH Blocking Solution, Cat. No. MB-1220). 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 minutes or longer at 37 °C.

Note: 5% nonfat dry milk plus 0.1% Tween 20 in 4x SSC can be used as an alternative blocking solution. (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.) 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 in 1x ISH Blocking Solution approximately 30 minutes 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 minutes at room temperature.

4. Wash slide for 2 x 3 minutes in 1x ISH Blocking Solution.

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 minutes at room temperature.

6. Wash slides for 2 x 3 minutes in 1x ISH Blocking Solution.

7. Follow with a second incubation of the same Fluorescein Avidin DCS solution (5 µg/ml) for 30 minutes at room temperature.

8. Wash slides 2 x 5 minutes in 4x SSC + 0.1% Tween 20 before coverslipping with any one of the following mounting media: Vectashield® Mounting Medium (Cat. No. H-1000), Vectashield® Mounting Medium with DAPI (Cat. No. H-1200), Vectashield® Mounting Medium with propidium iodide (Cat. No. H-1300), Vectashield® Hard•Set™ Mounting Medium (Cat. No. H-1400), or Vectashield® Hard•Set™ Mounting Medium with DAPI (Cat. No. H-1500).

Fluorescent Detection of Fluorescein Labeled ISH Probes

1. After hybridization with fluorescein labeled DNA/RNA probes, block tissue sections or chromosome spreads for ≥ 30 minutes in 1x ISH Blocking Solution (5x ISH Blocking Solution, Cat. No. MB-1220). 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 minutes or longer at 37 °C.

Note: 5% nonfat dry milk plus 0.1% Tween 20 in 4x SSC can be used as an alternative blocking solution. (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.) 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 in 1x ISH Blocking Solution for approximately 30 minutes before use to minimize non-specific binding.

3. Tip off the blocking solution and incubate with Biotinylated Anti-Fluorescein solution (10 µg/ml) for 30 minutes at room temperature.

4. Wash slides for 2 x 3 minutes in 1x ISH Blocking Solution.

5. Incubate with the Fluorescein Avidin DCS solution (10 µg/ml) for 30 minutes at room temperature.

6. Wash slides 2 x 5 minutes in 4x SSC + 0.1% Tween 20 before coverslipping with any one of the following mounting media: Vectashield® Mounting Medium (Cat. No. H-1000), Vectashield® Mounting Medium with DAPI (Cat. No. H-1200), Vectashield® Mounting Medium with propidium iodide (Cat. No. H-1300), Vectashield® Hard•Set™ Mounting Medium (Cat. No. H-1400), or Vectashield® Hard•Set™ Mounting Medium with DAPI (Cat. No. H-1500).

Chromogenic Detection of Biotin Labeled ISH probes

1. After hybridization with biotinylated DNA/RNA probes, block tissue sections or chromosome spreads for ≥ 30 minutes in 1x ISH Blocking Solution (5x ISH Blocking Solution, Cat. No. MB-1220). 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 minutes or longer at 37 °C.

Note: 5% nonfat dry milk plus 0.1% Tween 20 in 4x SSC can be used as an alternative blocking solution. (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.) 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 Alkaline Phosphatase Streptavidin (Cat. No. SA-5100) 1:200 - 1:1000 in 1x ISH Blocking Solution approximately 30 minutes before use to minimize non-specific binding.

3. Tip off the blocking solution and incubate with diluted Alkaline Phosphatase Streptavidin solution for 30 minutes at room temperature.

4. Wash slide for 2 x 3 minutes 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 Alkaline Phosphatase Streptavidin reagent at a dilution of approximately 1:2500.

6. Wash in 100 mM Tris, pH 9.5 buffer for 5 minutes.

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).

Chromogenic Detection of Fluorescein Labeled ISH Probes

1. After hybridization with fluorescein labeled DNA/RNA probes, block tissue sections or chromosome spreads for ≥ 30 minutes in 1x ISH Blocking Solution (5x ISH Blocking Solution, Cat. No. MB-1220). 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 minutes or longer at 37 °C.

Note: 5% nonfat dry milk plus 0.1% Tween 20 in 4x SSC can be used as an alternative blocking solution. (4x SSC is 0.6 M NaCl, 60 mM sodium citrate, pH 7.0.)

2. Dilute Alkaline Phosphatase Anti-Fluorescein (Cat. No. MB-2100) to 5 µg/ml in 1x ISH Blocking Solution approximately 30 minutes before use to minimize non-specific binding.

3. Tip off the blocking solution and incubate with diluted Alkaline Phosphatase Anti-Fluorescein solution for 30 minutes at room temperature.

4. Wash slide for 2 x 3 minutes 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 Alkaline Phosphatase Anti-Fluorescein reagent at a concentration of 0.2-2.0 µg/ml.

6. Wash in 100 mM Tris, pH 9.5 buffer for 5 minutes.

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).