Vysis IGH/MYC/CEP 8 Tri-Color DF FISH Probe Kit

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30-608467/R4

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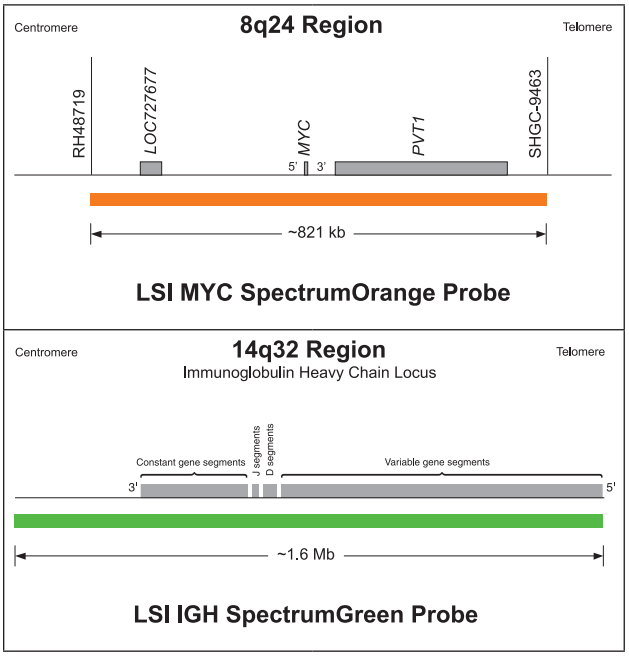
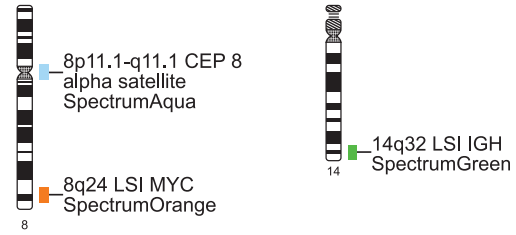
NOTE: Changes Highlighted

Key to Symbols Used

	Manufacturer
REF	Reference Number
LOT	Lot Number
IVD	In Vitro Diagnostic Medical Device
	Temperature Limitation
	Danger
	Biological Risks
	Caution, consult accompanying documents
	Use By
	Consult instructions for use
EC REP	Authorized Representative in the European Community





Intended Use

The Vysis LSI IGH/MYC/CEP 8 Tri-color Dual Fusion fluorescence in situ hybridization probes are intended to detect the t(8;14)(q24;q32) reciprocal translocation involving the IGH and MYC gene regions.

Summary and Explanation of the Test

The t(8;14)(q24;q32) translocation is a hallmark of Burkitt's Lymphoma (BL) and occurs in about 80% of BL cases.[1] As such, testing for t(8;14)(q24;q32) or variants is indicated as an essential test for BL.[2] The Vysis LSI IGH/MYC/CEP 8 Tri-color Dual Fusion probe has been used to identify the t(8;14)(q24;q32) translocation in published reports.[3,4] The aqua CEP 8 probe serves as a control for the copy number of chromosome 8.

Probe Description

The SpectrumOrange probe spans approximately 821 kb (chr8:128432540-129253747; March 2006 assembly)[5] and covers the MYC gene region.

The SpectrumAqua probe contains D8Z2 alpha satellite sequences and is specific to chromosome 8p11.1-q11.1.

The approximately 1.6 Mb (chr14:104736507-106339460; March 2006 assembly)[5] SpectrumGreen probe spans the IGH region.

Reagents

1. Vysis LSI IGH/MYC/CEP 8 Tri-Color Dual Fusion Probes (Part No. 30-191020)

(1 vial, 20 µL per vial). 1025 ng/µL, Fluorophore-labeled DNA probes and blocking DNA in Tris-EDTA.

2. Vysis LSI/WCP Hybridization Buffer (Part No. 30-804826)

(1 vial, 150 µL per vial). Dextran sulfate, formamide, SSC (pH 7.0).

Storage Instructions

-15°C
-25°C

The Vysis IGH/MYC/CEP 8 Tri-Color DF FISH Probe Kit must be stored at −20°C (±5°C), protected from light.

Shipping Conditions

The Vysis IGH/MYC/CEP 8 Tri-Color DF FISH Probe Kit is shipped on dry ice.

If you receive reagents that are in a condition contrary to label recommendation, or that are damaged, contact Abbott Molecular Technical Services.

Warnings and Precautions

IVD In Vitro Diagnostic Medical Device

For In Vitro Diagnostic Use

Vysis LSI IGH/MYC/CEP 8 Tri-Color Dual Fusion Probes

CAUTION: This preparation contains human sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[7] OSHA Standards on Bloodborne Pathogens,[8] CLSI Document M29-A3,[9] and other appropriate biosafety practices.[10] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[7]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[10]

Vysis LSI/WCP Hybridization Buffer

Danger

Hazard-determining components of labeling: Formamide

H360	May damage fertility or the unborn child.
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P281	Use personal protective equipment as required.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.
P501	This material and its container must be disposed of in a safe way.

Safety Data Sheet Statement: Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet.

NOTE: Material Safety Data Sheets (MSDS) for all reagents provided in the kits are available upon request from the Abbott Molecular Technical Services Department.

Preparing the Reagents

NOTE: Where indicated, measure the pH of these solutions at ambient temperature. Use a pH meter with a glass electrode unless otherwise noted.

20X SSC Solution

Mix thoroughly 132 g 20X SSC in 400 mL purified water. Measure pH and adjust to pH 5.3 with HCl. Add purified water to bring final volume to 500 mL. Mix and filter through a 0.45 µm filtration unit. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

2X SSC/0.1% NP-40 Wash Solution

Mix thoroughly 100 mL 20X SSC solution (pH 5.3) with 850 mL purified water. Add 1 mL NP-40. Mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust to pH 7.0 ± 0.2 with NaOH. Add purified water to bring final volume to 1 liter. Mix and filter through a 0.45 µm filtration unit. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

Denaturation Solution (70% Formamide/2X SSC)

Mix thoroughly 49 mL formamide, 7 mL 20X SSC solution (pH 5.3) and 14 mL purified water in a glass Coplin jar. Using pH indicator strips, verify that pH is between 7.0 and 8.0. Between uses, store covered at 2 to 8°C. Discard after 7 days.

Ethanol Solutions (70%, 85%, 100%)

Prepare v/v dilutions of 100% ethanol with purified water. Between uses, store covered at ambient temperature. Discard stock solutions after 6 months.

2X SSC/0.3% NP-40 Wash Solution

Mix thoroughly 100 mL 20X SSC solution (pH 5.3) with 850 mL purified water. Add 3 mL of NP-40. Mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust pH to 7.0 to 7.5 with NaOH. Add purified water to bring final volume of the solution to 1 liter. Mix and filter through a 0.45 µm filtration unit. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

Storage of the LSI DNA Probe: The LSI DNA Probe should be stored at −20°C (±5°C), protected from light.

Degradation: Fluorophores are readily photobleached by exposure to light. To limit this degradation, handle all solutions and slides containing fluorophores in reduced light. Perform all steps that do not require light for manipulation, such as incubations and washes, in darkness.

Procedural Notes: Prior to use, thaw reagents at ambient temperature, then centrifuge each tube 2 to 3 seconds using a standard bench-top microcentrifuge.

Specimen Collection and Preparation for Analysis

Formalin-fixed, paraffin-embedded tissue specimens should be placed on slides using standard procedures. To prepare paraffin-embedded samples for FISH, sample should be deparaffinized and pretreated to maximize tissue permeability and hybridization using standard procedures.

Procedure

Materials Required

- Vysis LSI IGH/MYC/CEP 8 Tri-color Dual Fusion Probe
- Vysis LSI/WCP Hybridization Buffer

Materials Required But Not Provided

- 12N HCl (for adjusting pH of wash solutions)
- 1N NaOH (for adjusting pH of wash solutions)
- Glass Coplin jars
- Calibrated thermometer
- Forceps
- Graduated cylinder (1000 mL)
- Magnetic stirrer
- Ethanol
- Microcentrifuge
- Microliter pipette tips for 1 to 10 µL volumes
- Microliter pipettor for 1 to 10 µL volumes
- pH meter
- Precleaned glass microscope slides
- Purified water
- Timer
- Vortex mixer
- Water bath (37°C and 72°C)
- Fluorescence microscope
- 37°C incubator
- 20X SSC
- NP-40
- Formamide, Ultra Pure
- DAPI II Counterstain
- Slide warmer

Prepare 3 Coplin jars: Pour 70 mL 100% ethanol into 1 jar, 70 mL 85% ethanol into another, and 70 mL 70% ethanol into the last. Use at ambient temperature. Discard after 7 days or if excessive dilution or evaporation has occurred.

For optimal results, ensure that reagents are made and used at the temperatures described in this package insert.

Measure the temperatures of the solutions inside the Coplin jar; use of a calibrated thermometer is required.

Preparing the Specimen Target

NOTE: Bring Coplin jars containing the denaturation solution to ambient temperature. Place jars in a 73 ± 1°C water bath approximately 30 minutes prior to use to bring the solution to temperature.

1. Ensure that the temperature of the denaturation solution is 73 ± 1°C. To maintain the proper temperature of the denaturing solution, immerse 4 slides simultaneously. If you have less than 4 slides, add blank slides that are at ambient temperature to bring the total to 4.
2. Immerse the slides in the denaturation solution for 5 minutes.

   NOTE: Immerse no more than 4 slides in the Coplin jar simultaneously.

3. Dehydrate slides for 1 minute in 70% ethanol, followed by 1 minute in 85% ethanol, and 1 minute in 100% ethanol.

NOTE: Keep the slides in 100% ethanol until ready to dry all slides and apply the probe mixture.

Preparing the Probe Mixture

1. For each target area, add the following to a microcentrifuge tube at ambient temperature:
   7 µL LSI/WCP Hybridization Buffer
   1 µL probe
   2 µL purified water

   NOTE: To simultaneously hybridize up to 3 probes, each labeled with a different fluorophore, add 1 µL of each probe. Add purified water to bring the combined volume of probe and water to 3 µL.
2. Centrifuge tube for 1 to 3 seconds.
3. Vortex and centrifuge again.
4. Place tubes in a 73±1˚C water bath for 5 minutes.
5. Remove tube from water bath.
6. Place tube on a 45 to 50˚C slide warmer until ready to apply probe to target DNA.

   NOTE: If slides are ready when probe is denatured, you can apply probe immediately to target DNA.

Hybridizing the Probe to the Specimen Target

NOTE: Prepare a humidified box by placing a paper towel moistened with water on the side of an airtight container. Place in 37˚C incubator.

1. Remove the slides from the 100% ethanol.
2. Dry slides by touching the bottom edge of the slides to a blotter and wiping the underside of the slides dry with a paper towel.
3. Place slides on a 45 to 50˚C slide warmer to evaporate remaining ethanol.
4. Apply 10 µL of probe mixture to 1 target area and immediately apply coverslip. Repeat for additional target areas.
5. Seal coverslip with rubber cement.
6. Place slides in a prewarmed humidified box and place box in a 37˚C incubator for 6 to 16 hours.

To produce an assay with sufficient signal, start with a 12- to 16-hour hybridization for most LSI probes.

Washing the Slide

Prepare the wash solutions:

- Pour 70 mL of 2X SSC/0.3% NP-40 into a Coplin jar. Place jar in a 73±1˚C water bath at least 30 minutes prior to use. Use 1 day, then discard.
- Pour 70 mL of 2X SSC/0.1% NP-40 into a Coplin jar. Use at ambient temperature. Use 1 day, then discard.

NOTE: To maintain the proper temperature in 2X SSC/0.3% NP-40, wash 4 slides simultaneously. If you have less than 4 slides, add blank slides that are at ambient temperature to bring the total to 4.

Start timing when the fourth slide is immersed.

1. Remove the rubber cement from 1 slide while minimally disturbing the cover slip, and immerse the slide in 2X SSC/0.1% NP-40. Repeat with other slides and let stand 2 to 5 minutes to allow coverslips to float off slides.
2. Immerse the slide in the 2X SSC/0.3% NP-40. Agitate slides for 1 to 3 seconds. Repeat with other slides.
3. Remove slides after 2 minutes.

   NOTE: Ensure the temperature of the wash solution is 73±1˚C before washing another 4 slides.
4. Immerse slides in 2X SSC/0.1% NP-40. Agitate slides for 1 to 3 seconds. Remove slides after 5 seconds to 1 minute.

Visualizing the Hybridization

1. Air-dry slide in darkness.
2. Apply 10 µL of DAPI II counterstain to the target area of slide and apply coverslip.

View slides using a suitable filter set on an optimally performing fluorescence microscope. The following optical filter sets will visualize the fluorophores used in the hybridization.

Using this Vysis filter…	Allows simultaneous excitation and emission of…
DAPI/Orange	DAPI and SpectrumOrange fluorophores
DAPI/Green	DAPI and SpectrumGreen fluorophores
Aqua/Green/Orange	SpectrumAqua, SpectrumGreen, and SpectrumOrange fluorophores
DAPI/Orange/Green	DAPI, SpectrumOrange, and SpectrumGreen fluorophores
DAPI/Aqua/Green/Orange	DAPI, SpectrumAqua, SpectrumGreen, and SpectrumOrange fluorophores

Storage: Slides stored at −20˚C (±5˚C) protected from light can be analyzed for at least 3 weeks after hybridization.

Using Codenaturation

Codenaturation is a process that simplifies FISH by combining denaturation of probe mixture and specimen into a single step. Typically, codenaturations are performed by placing the specimen slides, with probes and coverslips applied, on the surface of a hot plate or the shelf of an oven or incubator that is at the denaturation temperature. Generally, after 2 to 10 minutes, the slides are removed and placed in an incubator set at the hybridization temperature.

Published conditions for codenaturation specify a broad range of temperatures and times, reflecting the need to optimize conditions for specific applications and specimen types. The parameters described here are for use with the Vysis ThermoBrite Denaturization/Hybridization System and are intended to provide a set of starting parameters. Further optimization may be required depending on the specimen. The appearance of a hybridization using codenaturation may vary from a hybridization where the specimen target is denatured and dehydrated before the probe is applied.

Preparing a Slide for Codenaturation

1. For each target area, add the following to a microcentrifuge tube at ambient temperature:
   7 µL LSI/WCP Hybridization Buffer
   1 µL probe
   2 µL purified water

   NOTE: To simultaneously hybridize up to 3 probes, each labeled with a different fluorophore, add 1 µL of each probe. Add purified water to bring the combined volume of probe and water to 3 µL.
2. Centrifuge tube for 1 to 3 seconds.
3. Vortex and then centrifuge again.
4. Apply 10 µL of probe mixture to a slide and immediately apply coverslip.
5. Seal coverslip with rubber cement.

Setting the Denaturation/Hybridization System Parameters

The directions below are intended to be starting parameters for the ThermoBrite Denaturization/Hybridization System. Please refer to the ThermoBrite Operator's Manual for more information about how to use this instrument.

When using the ThermoBrite system, it may be necessary to adjust the denaturation and hybridization conditions. For further guidance, please refer to the troubleshooting section of this insert.

1. For cultured lymphocytes and bone marrow, set the Melt Temp to 73˚C and the Melt Time to 1 minute. For paraffin-embedded samples, set the Melt Temp to 73˚C and the Melt Time to 5 minutes.
2. Set the Hyb Temp to 37˚C and the Hyb Time to between 4 hours and overnight.
3. When the hybridization time is completed, wash the slides using the rapid wash procedure.
4. Air-dry slide in darkness.
5. Apply 10 µL counterstain to the target area and apply coverslip.

Quality Control Procedures

Positive and negative controls should be run with patient specimens.

Limitations of Use

For interphase use, individual laboratories should establish an analytical normal cut-off for the abnormal signal pattern of interest.[6]

Expected Results

The expected normal signal pattern of the Vysis LSI IGH/MYC/CEP 8 Tri-color Dual Fusion Probes is 2 orange signals, 2 green signals, and 2 aqua signals.

The expected abnormal pattern of the Vysis LSI IGH/MYC/CEP 8 Tri-color Dual Fusion Probes is 1 orange, 1 green, 2 fusions, and 2 aqua signals. The aqua CEP 8 probe serves as a control for the copy number of chromosome 8. Other abnormal signal patterns may occur and metaphase analysis may be helpful in characterization of such patterns.

Troubleshooting Results from a Codenaturation Assay

The chromosome morphology observed in a hybridization where codenaturation is used may differ from a specimen that is denatured and dehydrated before the probe is applied.

Problem	Possible Solution
Cross-hybridization	Repeat the assay on a new specimen using 1 of the following: • Increase the temperature of 2X SSC/0.3% NP-40 by 2°C. As needed, continue to increase the temperature until the signal intensity becomes acceptable. • Decrease the melt temperature by 2°C.
Probe appears dim	Repeat the assay on a new specimen using 1 of the following: • Increase the hybridization time. • Increase the melt temperature. As needed, continue to increase the temperature until the morphology becomes acceptable. • Wash the slide using 2X SSC/0.3% NP-40 at 70 to 73°C.
Diffuse signal (speckling)	Repeat the hybridization using 1 of the following: • Decrease the melt temperature 2°C. • Decrease the melt time. **NOTE: As needed, reduce the melt temperature or time until signal intensity becomes acceptable.** • Wash using the 2X SSC/0.3% NP-40 at 73 to 76°C.
Poor metaphase morphology	Repeat the assay on a new specimen using 1 of the following: • Decrease the melt temperature 2°C. • Decrease the melt time. **NOTE: As needed, reduce the melt temperature or time until signal intensity becomes acceptable.** • Pretreat the slides: 1. Prepare a solution of 2X SSC/1% paraformaldehyde. 2. Immerse the slide in 2X SSC/1% paraformaldehyde for 1 minute. 3. Immerse the slide several times in purified water. 4. Dehydrate slides through a series of 1 minute ethanol rinses (70%, 85%, 100%). 5. Air-dry the slides and continue with **Preparing a Slide for Codenaturation**.
Poor metaphase morphology	• If poor morphology persists, modify use of the ThermoBrite instrument: 1. Prepare 280 µL 70% formamide/2X SSC denaturation solution (196 µL formamide/28 µL 2X SSC/56 µL purified water). 2. Run a ThermoBrite Hold Temp program set at 73°C. 3. Place 10 µL of 70% formamide/2X SSC denaturation solution on each target area and coverslip. 4. When the ThermoBrite reaches 73°C, place the slides on the heating surface. Close the cover. 5. Remove slides after 3 minutes. 6. Remove coverslip. 7. Continue with the dehydration step in **Preparing the Specimen Target** for the non-codenaturation assay procedure.

Tips and Troubleshooting

When viewing the results of a FISH assay, ensure that your microscope is properly aligned and functioning optimally.

The following table lists some less than optimal results that you may encounter using the LSI probes. Probable causes and suggestions to improve assay performance are included.

Problem	Probable Cause	Possible Solution
Distorted chromosome morphology	Specimen slides dried too quickly during preparation.	Increase temperature of water bath (increases humidity) used when dropping slides. Decrease the temperature of the slide warmer during sample preparation. Increase drying time to at least overnight at ambient temperature, and then age slides at least 24 hours at ambient temperature. Do not bake slides at high temperature.
	Specimen over-denatured.	Ensure the denaturation solution was made according to the package insert. Ensure temperature of denaturation solution is 73±1°C prior to immersing the slide; decrease the temperature to 72°C. Decrease the time the slide is immersed in the denaturation solution by 1 to 3 minutes.
	Specimen slides not thoroughly dry prior to immersion in denaturation solution.	Warm specimen slides to 45 to 50°C prior to denaturation or dehydrate slides through a series of 1 minute ethanol rinses (70%, 85%, 100%).
	Specimen slides too fresh prior to denaturation.	Age slides at least 24 hours at ambient temperature.
High slide background	Glass slides not sufficiently cleaned prior to sample preparation.	Immerse glass slides in ethanol and wipe dry using lint-free paper prior to dropping slides.
	Cellular debris in sample preparation.	Wash cell pellet with fresh fixative 3 times and repeat the slide dropping procedure.
	Metaphase spreads were aged by baking or contain cytoplasm.	Increase time the slide is immersed in the denaturation solution to 10 minutes.

Problem	Probable Cause	Possible Solution
High slide background	Slide inadequately washed following hybridization.	Ensure the wash solutions were made according to the package insert.
		Ensure pH and temperature of wash solutions are correct.
		Remove coverslip. Repeat the wash procedure.
	Wash solutions used too long or stored improperly.	Ensure wash solutions containing formamide are stored at 2 to 8˚C. Discard after 7 days or frequent use. Discard all other wash solutions after 1 day.
		Ensure the pH of the formamide wash solutions are pH 7.0 to 8.0.
	Viewed hybridization using long bandpass filters.	Switch to filters with smaller bandwidths or to multi-bandpass filters to reduce background light.
Weak or no signal	Specimen slide not adequately denatured.	Ensure temperature of denaturation solution in the Coplin jar is 73±1˚C prior to immersing the slide.
		Increase temperature of denaturation solution to 74˚C.
		Increase the time the slide is immersed in the denaturation solution by 2 to 4 minutes.
	Specimen slide not adequately prepared for FISH.	Contact Abbott Molecular Technical Services for protocols describing how to prepare a specimen for FISH.
	Specimen slides improperly aged after dropping specimen.	Age for 24 hours at ambient temperature before performing FISH on them.
	Specimen slides not thoroughly dry prior to immersion in denaturation solution.	Warm specimen slides to 45 to 50˚C prior to denaturation or dehydrate slides through a series of 1 minute ethanol rinses (70%, 85%, 100%).
	Specimen was GTG-banded.	Use of trypsin-Giemsa banded specimens for FISH may require adjustments in banding and/or hybridization protocols. For further information on banding prior to FISH, contact Abbott Molecular Technical Services or prepare fresh specimen slides.
	Probe not added.	Prepare a new probe mixture. Allow the probe to thaw completely. Vortex or pipet reagents to mix; centrifuge briefly. Pipet probe slowly.
	Probe, hybridization buffer, or probe mixture were not mixed well prior to use.	Vortex or pipet reagents to mix; centrifuge briefly.
	Probes improperly diluted for hybridization.	Use the volumes stated in the assay procedure to maintain the ratio of the probe mix (7 µL hybridization buffer: 1 µL probe: 2 µL purified water).
		Ensure the pipet is calibrated.
		Allow hybridization buffer to thaw completely and to reach ambient temperature prior to use; pipet slowly.
Weak or no signal	Probe not adequately denatured. **NOTE: Does not apply to probes that are supplied in hybridization buffer and denatured.**	Ensure temperature of the water bath used to denature the probe mix is 73±1˚C.
		Denature the probe mixture for 5 minutes.
	Probe not applied to the target sample immediately after the probe was denatured.	Plan so the probe is applied immediately after the slides are removed from the 100% ethanol solution. Ensure the ethanol has evaporated before applying probe.
		Remove tube containing probe mix from 73±1˚C water bath and immediately place the tube on a 45 to 50˚C slide warmer. Keep the tube on the slide warmer while pipetting the probe onto the slide.
		Process only as many slides as you can and still maintain the correct temperatures and times according to the assay procedure.
	Probe mix dried too much on the specimen slide.	Immediately place the coverslip over the target area after applying probe mix.
		When washing the hybridization, remove the coverslip from 1 slide at a time and immediately immerse the slide into the wash solution before removing the coverslip from the next slide.
	Air bubbles were trapped under the coverslip during hybridization.	Apply coverslip by first touching the surface of the probe mixture.
		Place the slide with the coverslip down on a blotter and very gently press out visible bubbles.
	Hybridization conditions inappropriate.	Ensure that the stated time and temperatures for the hybridization were followed.
		Ensure that the temperature of the incubator is 37˚C.
		Seal the coverslip well with rubber cement, leaving no gaps.
		Increase the hybridization time.
	Wash conditions or solutions incorrect.	Ensure that the wash solutions were made according to the package insert.
		Ensure the temperatures of the wash solutions are at the stated temperatures for the wash procedure followed.
		Ensure that the thermometers and pH meters used are calibrated properly.
		Remove coverslips before immersing slides in wash solution.
	Probes or specimen slides stored improperly.	Store undiluted probe at −20˚C (±5˚C), protected from light.
		Store non-hybridized slides desiccated at −20˚C (±5˚C) for an extended period or at ambient temperature for short periods.
		Store hybridized slides at −20˚C (±5˚C), protected from light for up to 3 weeks.

Problem	Probable Cause	Possible Solution
Weak or no signal	Wrong counterstain used.	Remove coverslip. Immerse slides for 5 minutes in 2X SSC/0.1% NP-40 at ambient temperature; dehydrate slide through a series of 1 minute ethanol rinses (70%, 85%, 100%). Air-dry and reapply counterstain.
	Counterstain is too bright.	
	Viewed hybridization using inappropriate filter set.	Multi-bandpass filter sets provide less light than single bandpass filter sets, so probe signals may appear fainter when viewed through the multi-bandpass sets.
		Use correct filter for viewing the probe fluorophore. Contact Abbott Molecular Technical Services for further information.
	Microscope configuration or objectives not adequate for viewing FISH results, or microscope filters are damaged.	Contact your microscope manufacturer.
Low signal specificity	Probes diluted inappropriately; often too much probe in the assay.	Ensure the probe mixture was made according to the package insert.
	Inappropriate hybridization conditions.	Ensure temperature of incubator is 37˚C.
		Ensure that the hybridization buffer was added to the probe mixture and in the proper amount.
	Wash temperature too low.	Maintain the wash temperature of the wash solutions by placing no more than 4 slides in 1 Coplin jar at a time and ensuring that the temperature of the wash solution is correct before washing another set of slides.
	Wash solution stringency too low.	Ensure the wash solutions were made according to the package insert.
		NOTE: The lower the concentration of salt (SSC), the higher the concentration of formamide and NP-40, the more stringent the wash.
Bright or weak counterstain	Counterstain appears weak: specimen slides not dehydrated prior to applying counterstain or oil droplets in counterstain.	Remove coverslip. Immerse slides for 5 minutes in 2X SSC/0.1% NP-40 at ambient temperature; dehydrate slide through a series of 1 minute ethanol rinses (70%, 85%, 100%). Air dry and reapply counterstain.
	Wrong concentration of counterstain.	If counterstain appears too bright, dilute the counterstain in antifade solution (List No. 06J29-010) before applying.
	NOTE: DAPI I counterstain is 8 times more concentrated than DAPI II counterstain.	
	Counterstain too old or exposed to light for extended periods.	Store counterstain at −20˚C (±5˚C), protected from light and when using.
		Ensure the counterstain is not used past the expiration date.

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Technical Assistance

For technical assistance, call Abbott Molecular Technical Services at +49-6122-580 or visit the Abbott Molecular Web site at http://www.abbottmolecular.com.

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