Vysis LSI (Locus Specific Identifier) DNA Probe FISH Procedure

The LSI probes consist of DNA probe sequences homologous to specific DNA regions, gene sequences or loci and are directly labeled with one of the Vysis fluorophores. Unlabeled blocking DNA is included with the probe to suppress sequences contained within the loci that are common to other chromosomes. When hybridized and visualized, these probes show specific changes, such as amplifications, deletions or translocations, to specific genes, loci, or chromosomal regions.

Some LSI probes are premixed with the LSI/WCP Hybridization Buffer and packaged in quantities of 200 µL.

Material Safety Data Sheets (MSDS) on all reagents provided are available from the Abbott Molecular Technical Service Department.

General Purpose Reagents

Reagent	Vysis Order No./ Abbott Order No.	Package Size
20X SSC	32-804850/02J10-032	500g
DAPI II Counterstain	32-804831/06J50-001	500 µL x 2 (125 ng/mL)
DAPI I Counterstain	32-804830/06J49-001	500 µL x 2
NP-40	32-804818/07J05-001	1000 µL x 2
Propidium Iodide Counterstain	32-804829/07J06-001	500 µL x 2 (400 ng/mL)
LSI/WCP Hybridization Buffer	32-804826/06J67-001	150 µL x 2
LSI/WCP Hybridization Buffer	NA/06J67-011	500 µL x 2
100% Ethanol (EtOH)		
12N HCl		
1N NaOH		
formamide, ultrapure grade		
purified water (distilled or deionized)		

Warning & Precautions: Fluorophores are readily photobleached by exposure to light. To limit this degradation, handle all solutions and slides containing fluorophore labeled probes in reduced light.

FISH Procedure

The following procedure has been validated for performance on cultured peripheral blood lymphocytes and is used to determine the probe quality. The user is responsible for validating the procedure for their specific application.

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 132g 20X SSC in 400 mL purified H_2O. Measure pH and adjust to pH 5.3 with HCl. Add purified H_2O to bring final volume to 500 mL. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

0.4X SSC / 0.3% NP-40 wash solution

Mix thoroughly 20 mL 20X SSC (pH 5.3) with 950 mL purified H_2O. Add 3 mL of NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust pH to 7.0-7.5 with NaOH. Add purified H_2O to bring final volume of the solution to 1 liter. 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 (pH 5.3) with 850 mL purified H_2O. Add 1 mL NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust to pH 7.0±0.2 with NaOH. Add purified H_2O to bring final volume to 1 liter. Store at ambient temperature. Discard stock solution after 6 months, or sooner if solution appears cloudy or contaminated.

2X SSC / 0.3% NP-40 wash solution

Mix thoroughly 100 mL 20X SSC (pH 5.3) with 850 mL purified H_2O. Add 3 mL NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH and adjust to pH 7.0±0.2 with NaOH. Add purified H_2O to bring final volume to 1 liter. 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 (pH 5.3) and 14 mL purified H_2O in a glass Coplin jar. Measure pH using pH indicator strips to verify pH is 7.0-8.0. Between uses, store covered at 2-8°C. Discard after 7 days.

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

Prepare v/v dilutions of 100% ethanol with purified H_2O to make stock solutions of 75% and 85% ethanol. Between uses, store covered at ambient temperature. Discard stock solutions after 6 months.

For working solutions, pour 70 mL 100% EtOH into one of three jars; 70 mL 85% EtOH into another, and 70 mL 70% EtOH into the last. Use at ambient temperature. Discard after 7 days or if excessive dilution or evaporation has occurred.

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

For good results, ensure that reagents are made and used at the temperatures described herein.

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

NOTE: Any Hybridization that includes an LSI probe should use this procedure.

Preparing the Specimen Target

NOTE: For formalin fixed paraffin embedded (FFPE) specimens or cytology specimens containing cells of epithelial origin, contact technical services for information pretaining to pretreatments.

NOTE: Bring Coplin jars containing the denaturation solution to ambient temperature. Place jars in a 74±1°C water bath approximately 30 minutes prior to use to bring the solution to temperature.

1. Mark hybridization areas with a diamond tipped scribe on the underside of the specimen slide.
2. Ensure that the temperature of the denaturation solution is 73±1°C.
3. Immerse the slides in the denaturation solution for 5 minutes.
   NOTE: Immerse no more than four slides in the Coplin jar simultaneously.
4. Dehydrate slides for 1 minute in 70% EtOH, followed by 1 minute in 85% EtOH, and 1 minute in 100% EtOH.
   NOTE: Keep the slides in 100% EtOH until you are ready to dry all slides and apply the probe mixture.

Preparing the Probe Mixture

NOTE: If using a probe mixed with the LSI Hybridization Buffer, bring the probe to ambient temperature. Continue below to **Hybridizing the Probe to the Specimen Target**.

1. Add the following, for each target area, to a microcentrifuge tube at ambient temperature:
   7 µL LSI/WCP Hybridization Buffer
   1 µL probe
   2 µL purified H_2O
   NOTE: For probes labeled with different fluorophores, up to three may be added at 1µL each. The total volume of probe and H_2O should not exceed 3 µL, H_2O is not necessary if mixing three probes.
2. Centrifuge tube for 1-3 seconds.
3. Vortex and then centrifuge again.
4. Place tube in a 73±1°C water bath for 5 minutes.
5. Remove tube from water bath.
6. Place tube on a 45-50°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: The total time that the slide is on the warmer should not exceed 2 minutes.

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% EtOH.
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 45-50°C slide warmer to evaporate remaining EtOH or air dry the slides.
4. Apply 10 µL of probe mixture to one 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 6-16 hours. To produce an assay with sufficient signal, start with a 12-16 hour hybridization for most LSI probes.

Washing the Slide

NOTE: For samples that are paraffin-embedded sections or cytology specimens containing cells of epithelial origin substitute 2X SSC/0.3% NP-40 wash solution for the 0.4X SSC/0.3% NP-40 wash solution. A room temperature wash is not needed for this specimen type.

Prepare the wash solutions:

Pour 70 mL of 0.4X SSC/0.3% NP-40 into a Coplin jar. Place jar in a 74±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 0.4X SSC/0.3% NP-40, wash four slides simultaneously. If you have less than four slides, add blank slides that are at ambient temperature to bring the total to four.

Start timing when the fourth slide is immersed.

1. Remove coverslip from one slide and immediately immerse the slide in the 0.4X SSC/0.3% NP-40. Agitate slides for 1-3 seconds. Repeat with other slides.
2. Remove slides after 2 minutes.
   NOTE: Ensure the temperature of the wash solution is 73±1˚C before washing another four slides.
3. Immerse slides in 2X SSC/0.1% NP-40. Agitate slides for 1-3 seconds. Remove slides after 5 seconds to 1 minute.

Visualizing the Hybridization

1. Air dry slide in darkness.
2. Apply 10 µL counterstain to the target area of slide and apply coverslip. For the evaluation of SpectrumGreen and/or SpectrumAqua probes you can use DAPI I, DAPI II or PI counterstains; For the evaluation of individual probes (or combination of probes including) SpectrumOrange, SpectrumGold or SpectrumRed probes only use DAPI I or DAPI II counterstain.

View hybridized 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 . . .**
Aqua	SpectrumAqua fluorophores
DAPI	DAPI
Gold	SpectrumGold fluorophores
Green or Green(V.2)	SpectrumGreen fluorophores (Green[V.2] is recommended when a SpectrumGreen probe is used in the same mix as a SpectrumGold probe)
Red	SpectrumRed fluorophores
Red/Green	SpectrumRed and SpectrumGreen fluorophores
DAPI/Red/Green	DAPI, SpectrumRed and SpectrumGreen fluorophores
DAPI/Green	DAPI and SpectrumGreen fluorophores
DAPI/Orange	DAPI and SpectrumOrange fluorophores
DAPI/Orange/Green	DAPI, SpectrumOrange and SpectrumGreen fluorophores
DAPI/Orange/Green (V.2)	DAPI, SpectrumOrange, and SpectrumGreen fluorophores are recommended when SpectrumOrange LSI Probes are mixed with SpectrumGreen CEP Probes.

Storage

Store hybridized slides, with coverslip and counterstain, at -20˚C in the dark.

Using Codenaturation

Codenaturation is a process that simplifies fluorescence *in situ* hybridization (FISH) by combining denaturation of probe mixture and specimen into a single step. Typically, codenaturations are performed by placing the specimen slides with probe mix and coverslips applied and sealed, on the surface of the Vysis HyBrite or ThermoBrite® Denaturation/Hybridization Systems at the denaturation 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 are described in the user guides for Vysis HYBrite or ThermoBrite Denaturation/Hybridization Systems 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.

ThermoBrite is a trademark of Iris Sample Processing, Inc.

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