

# Vysis EGR1 FISH Probe Kit

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**REF** 4N37-020

30-608443/R3

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**NOTE: Changes Highlighted**

Key to Symbols Used	
	Reference Number
	Lot Number
	In Vitro Diagnostic Medical Device
	Use By
	Temperature Limitation
	Danger
	Danger
	Danger
	Danger
	Biological Risks
	Caution, consult accompanying documents
	Consult instructions for use
	Contains sufficient for <n> tests
	Manufacturer

## NAME

Vysis EGR1 FISH Probe Kit containing the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes

## INTENDED USE

The Vysis EGR1 FISH Probe Kit is intended to detect deletion of the LSI EGR1 probe target on chromosome 5q in bone marrow specimens and to be used, in addition to cytogenetics, other biomarkers, morphology and other clinical information, at the time of acute myeloid leukemia (AML) diagnosis as an aid in determining prognosis. Deletion of chromosome 5q has been associated with an unfavorable prognosis in AML patients.

## SUMMARY AND EXPLANATION OF THE TEST

Deletion of chromosome 5q as detected by cytogenetics is a recurring abnormality in AML. A commonly deleted segment on chromosome band 5q31 has been identified and the early growth response 1 (EGR1) gene is among the candidate genes in this segment.<sup>1</sup> A study suggests that haploinsufficiency of EGR1 may play a role in leukemogenesis.<sup>2</sup> The Vysis locus-specific identifier (LSI) EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes, components of the Vysis EGR1 FISH Probe Kit, have been used in several studies to detect EGR1 deletions.<sup>3-6</sup> A study, conducted as part of an Eastern Cooperative Oncology Group (ECOG) clinical trial, demonstrated the utility of the interphase fluorescence in situ hybridization (FISH) technique to stratify patients into cytogenetic risk categories at diagnosis of AML using the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes in conjunction with several additional FISH probes.<sup>3</sup> In addition, the prognostic importance of chromosome 5 abnormalities has been established in several large clinical studies.<sup>7,8</sup> The prognostic clinical utility of detecting specific chromosomal abnormalities in bone marrow specimens from patients diagnosed with AML is firmly established in both standard medical practice guidelines and in the medical literature. The National Comprehensive Cancer Network (NCCN) Practice Guidelines™ for Acute Myeloid Leukemia, which are the consensus recommendations of leading US AML experts, states that cytogenetics is the single most important prognostic factor in AML.<sup>9</sup> More specifically, the guidelines provide different risk categories for patients depending upon cytogenetic or molecular abnormalities. As an example, patients with deletion of chromosome 5q are in the 'poor risk' category and may have an unfavorable prognosis.

The Vysis EGR1 FISH Probe Kit uses FISH DNA probe technology to determine deletion status of the probe target for LSI EGR1, and the LSI D5S23, D5S721 probe serves as a control.

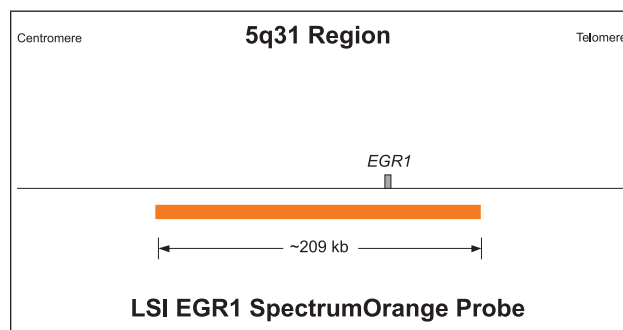
## BIOLOGICAL PRINCIPLES OF THE PROCEDURE

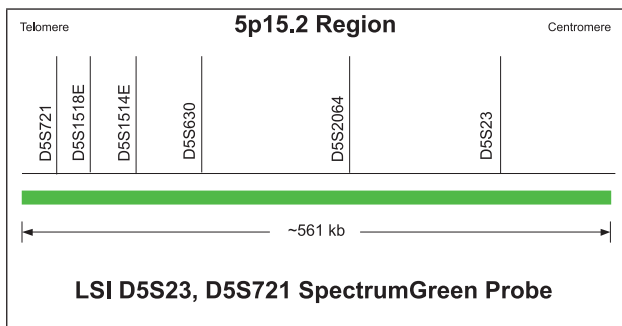
### DNA Probe Description

Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes

The SpectrumOrange-labeled LSI EGR1 probe, approximately 209 kb in length (chr5:137682107-137890637; March 2006 Assembly; UCSC Human Genome Browser<sup>10</sup>), is located at 5q31 and contains the complete EGR1 gene.

The SpectrumGreen-labeled LSI D5S23, D5S721 probe, approximately 561 kb in length (chr5:9450109-10011407; March 2006 Assembly; UCSC Human Genome Browser<sup>10</sup>), is located at 5p15.2.





## General Purpose Reagents Description

### Vysis LSI/WCP Hybridization Buffer

Vysis LSI/WCP Hybridization Buffer consists of dextran sulfate, formamide, and standard sodium citrate (SSC), which is used to promote rapid specific hybridization.

### DAPI II Counterstain

DAPI II Counterstain consists of DAPI (4',6-diamidino-2-phenylindole • 2HCl) (a DNA-specific fluorophore) and 1,4-phenylenediamine (an antifade compound used to reduce the tendency of the fluorophores to diminish in intensity) in a glycerol and phosphate buffered saline mixture.

### NP-40

NP-40 is a non-ionic surfactant that is used in the aqueous post-hybridization wash solution.

### 20X Standard Sodium Citrate (SSC) Salt

20X SSC is a salt composed of sodium chloride and sodium citrate. It is used to make 20X SSC solution and subsequent dilutions for denaturing and wash solutions.

### Technique Description

FISH is a technique that allows visualization of specific nucleic acid sequences within a cellular preparation. Specifically, FISH involves precise annealing of a single-stranded, fluorophore-labeled DNA probe to a complementary target sequence. Hybridization of the probe with the cellular DNA site is visible by direct detection using fluorescence microscopy. Interpretation of FISH results should be made utilizing appropriate controls and analytical techniques as well as taking into consideration other clinical and diagnostic test data.

Bone marrow cells from AML patients are attached to microscope slides using standard cytogenetic procedures. The resulting specimen DNA is denatured to single-stranded form and subsequently allowed to hybridize with the LSI EGR1 and LSI D5S23, D5S721 probes. Following hybridization, the unbound probe is removed by a series of washes, and the nuclei are counterstained with DAPI, a DNA-specific stain that fluoresces blue. Hybridization of the LSI EGR1 and LSI D5S23, D5S721 probes is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters, allowing visualization of the orange and green fluorescent signals.

In a cell with normal copy numbers of the LSI EGR1 and LSI D5S23, D5S721 probe targets, 2 SpectrumOrange signals (LSI EGR1) and 2 SpectrumGreen signals (LSI D5S23, D5S721) will be expected.

In a cell with the 5q deletion, one SpectrumOrange signal (LSI EGR1) and two SpectrumGreen signals (LSI D5S23, D5S721) will be expected. Enumeration of the orange LSI EGR1 and green LSI D5S23, D5S721 signals provide a mechanism for determining absolute copy number of the probe targets and the presence of the aberrations of interest.

## REAGENTS

### Materials Provided

This kit contains five reagents sufficient to process 20 assays. An assay is defined as one 22 mm × 22 mm LSI EGR1/D5S23, D5S721 DNA probe hybridization area.

### Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes

Part No.	30-171021
Quantity	1 vial, 20 µL/vial (100 and 300 ng/µL)
Storage	-20°C (± 10°C) and protected from light
Composition	SpectrumOrange and SpectrumGreen fluorophore-labeled DNA probes

### Vysis LSI/WCP Hybridization Buffer

Part No.	30-804824
Quantity	1 vial, 150 µL/vial
Storage	-20°C (± 10°C)
Composition	Dextran sulfate, formamide, and SSC

### DAPI II Counterstain

Part No.	30-804863
Quantity	1 vial, 600 µL/vial (125 ng/mL)
Storage	-20°C (± 10°C) and protected from light
Composition	DAPI (4',6-diamidino-2-phenylindole • 2HCl) in phenylenediamine dihydrochloride, glycerol, and buffer

### NP-40

Part No.	30-804821
Quantity	2 vials, 2000 µL/vial
Storage	-30°C to 30°C
Composition	NP-40 (non-ionic detergent)

### 20X SSC Salt

Part No.	30-805852
Quantity	1 bottle, 66 g
Storage	-30°C to 30°C
Composition	Sodium chloride and sodium citrate

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

## REAGENT STORAGE AND HANDLING INSTRUCTIONS

- The Vysis EGR1 FISH Probe Kit must be stored at -20°C (± 10°C) and protected from light when not in use.
- The NP-40 and 20X SSC Salt may be stored separately at room temperature.
- 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 only.

**CAUTION:** United States Federal Law restricts this device to sale and distribution to or on the order of a physician or to a clinical laboratory; use is restricted to, by, or on the order of a physician.

### Safety Precautions

**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,<sup>11</sup> OSHA Standards on Bloodborne Pathogens,<sup>12</sup> CLSI Document M29-A3,<sup>13</sup> and other appropriate biosafety practices.<sup>14</sup> 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.<sup>11</sup>
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.<sup>14</sup>

### Hazard-determining components of labeling:

- Formamide
- Polyethylene glycol octylphenyl ether

The following warnings apply:

**Danger**

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.
P280	Wear protective gloves / protective clothing / eye protection.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.
P501	Dispose of contents / container in accordance with local regulations.

**Danger**

H302	Harmful if swallowed.
H315	Causes skin irritation.
H318	Causes serious eye damage.
H411	Toxic to aquatic life with long lasting effects.
P280	Wear protective gloves / protective clothing / eye protection.



P264	Wash hands thoroughly after handling.
P273	Avoid release to the environment.
P305+P351 +P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P301+P312	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell.
P501	Dispose of contents / container in accordance with local regulations.

**Handling Precautions**

- The Vysis EGR1 FISH Probe Kit is only for use with specimens that have been handled and stored as described in this package insert.
- Do not use the Vysis EGR1 FISH Probe Kit beyond its Use By date.
- Package insert instructions must be followed. Failure to adhere to package insert instructions may yield erroneous results.

**Laboratory Precautions**

- All biological specimens should be treated as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all human specimens should be treated with universal precautions.
- Exposure of the specimens to acids, strong bases, or extreme heat should be avoided. Such conditions are known to damage DNA and may result in FISH assay failure.
- Failure to follow all procedures for slide denaturation, hybridization, and detection may cause unacceptable or erroneous results.
- The DAPI II Counterstain contains DAPI and 1,4-phenylenediamine.
- DAPI is a possible mutagen based on positive genotoxic effects. Avoid inhalation, ingestion, or contact with skin. Refer to MSDS for specific warnings.
- 1,4-phenylenediamine is a known dermal sensitizer and a possible respiratory sensitizer. Avoid inhalation, ingestion, or contact with skin. Refer to MSDS for specific warnings.
- Fluorophores are readily photo bleached by exposure to light. To limit this degradation, store slides and probe kits in the dark, and handle all slides and probe kits containing fluorophores in reduced light. This includes all steps involved in handling the hybridized slide. Carry out all steps which do not require light for manipulation (incubation periods, washes, etc.) in the dark or reduced light.
- The Vysis EGR1 FISH Probe Kit contains formamide, a teratogen. Avoid contact with skin and mucous membranes.
- Calibrated thermometers are required for measuring temperatures of solutions, water baths, and incubators.
- All hazardous materials should be disposed of according to the institution's guidelines for hazardous disposal.
- Prior to using the Vysis EGR1 FISH Probe Kit, verification of the normal cut-off is recommended.

**ASSAY PROCEDURE****Materials Provided**

- Vysis EGR1 FISH Probe Kit (List No. 4N37-020)

**Materials Required But Not Provided****Laboratory Reagents**

- Immersion oil appropriate for fluorescence microscopy
- Ethanol (EtOH), 100%. Store at room temperature.
- Purified water
- Rubber cement
- 12 N HCl
- 1 N NaOH

**Laboratory Equipment**

- Glass microscope slides, precleaned
- 22 mm x 22 mm glass coverslips
- Microliter pipettor (1 to 10 µL) and sterile tips
- Timer
- Microcentrifuge
- Graduated cylinders (100 to 1000 mL)
- Water baths (70 to 80°C)
- Water bath (37°C)
- Diamond-tipped scribe or solvent-resistant marker
- Forceps
- Disposable syringe (5 mL)
- Disposable pipettes (5 to 20 mL)
- Coplin jars (12 x 50 mL); Suggested type: Wheaton Product No. 900570 vertical staining jar
- Fluorescence microscope equipped with recommended filters (see **Microscope Equipment and Accessories** section)
- Calibrated thermometer
- Microscope slide box with lid and desiccant
- Magnetic stirrer
- pH meter
- Vortex mixer
- 0.45 µm filtration unit
- Slide warmer (54 to 58°C)
- ThermoBrite®
- ThermoBrite® Humidity Cards
- Cytogenetic drying chamber or fume hood
- Glass microscope slides stored in cold water
- Microcentrifuge tubes
- Methanol/acetic acid fixative
- Phase contrast microscope

**Microscope Equipment and Accessories**

**Microscope** An epi-illumination fluorescence microscope is required for viewing the hybridization results. The microscope should be checked to confirm it is operating properly to ensure optimum viewing of FISH assay specimens. A microscope used with general DNA stains such as DAPI, propidium iodide, and quinacrine may not function adequately for FISH assays. Routine microscope cleaning and periodic "tune-ups" by the manufacturer's technical representative, especially alignment of the lamp, if required, are advisable.

**Excitation Light Source** A 100 watt mercury lamp or other lamp with similar intensity and spectral output is the recommended excitation source. The manufacturer's technical representative should be consulted to assure that the fluorescence illumination system is appropriate for viewing FISH assay specimens. Record the number of hours that the bulb has been used and replace the bulb before it exceeds the rated time. Ensure that the lamp is properly aligned, if required.

**Objectives** Use oil immersion fluorescence objectives with numeric apertures  $\geq 0.75$  when using a microscope with a 100 watt mercury lamp or other lamp with similar intensity and spectral output. A 40X objective, in conjunction with 10X eyepieces, is suitable for scanning the specimen to select regions for enumeration. For enumeration of FISH signals, satisfactory results can be obtained with a 63X or 100X oil immersion achromat type objective.

**Immersion Oil** The immersion oil used with oil immersion objectives should be one formulated for low auto fluorescence and specifically for use in fluorescence microscopy.

**Filters** Hybridization of the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes to their target regions of the DNA is marked by orange and green fluorescence, respectively. The DNA which has not hybridized to the probes will fluoresce blue as a result of the DAPI II Counterstain.

The recommended filters for use with the Vysis EGR1 FISH Probe Kit are the Vysis Triple Band – DAPI, Green, and Orange Filter; the Vysis Dual Band (V2) – Green, Orange Filter; the Vysis Single Band Green Filter; and the Vysis Single Band Orange Filter.

## ASSAY PROTOCOL

Refer to the **WARNINGS AND PRECAUTIONS** section of this package insert before preparing samples.

### Working Reagent Preparation

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

Prepare v/v dilutions of 70% and 85% ethanol using 100% ethanol and purified water. Store the prepared solutions at room temperature in tightly capped containers. Dilutions may be used for up to one week unless evaporation occurs or the solution becomes diluted or cloudy due to excessive use.

#### 20X SSC Solution

Dissolve 66 g of 20X SSC salt using 200 mL of purified water in a suitable vessel. Measure pH at room temperature using a pH meter and adjust to pH 5.3 using 12 N HCl, if necessary. Transfer solution to a graduated cylinder and add purified water until a final volume of 250 mL is reached. Mix and filter the prepared solution through a 0.45 µm filtration unit. Store prepared stock solution at room temperature. Discard the stock solution after 6 months or sooner if solution appears cloudy or contaminated.

#### 2X SSC Solution

Mix thoroughly 100 mL of 20X SSC with 850 mL purified water in a suitable vessel. Measure pH at room temperature using pH meter to verify pH is  $7.0 \pm 0.2$ . If necessary adjust the pH using 1N NaOH. Add purified water to bring final volume of the solution to 1 L. Mix and filter through a 0.45 µm filtration unit. Store prepared stock solution at room 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 Solution and 950 mL purified water in a suitable vessel. Add 3 mL of NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH at room temperature using a pH meter and adjust pH to 7.0 to 7.5 with 1 N NaOH. Add purified water to bring final volume of the solution to 1 L. Mix and filter through a 0.45 µm filtration unit. Store prepared stock solution at room temperature. Discard stock solution after 6 months or sooner if solution appears cloudy or contaminated. Discard solution that was used in the assay at the end of each day.

#### 2X SSC/0.1% NP-40 Wash Solution

Using a suitable vessel, mix thoroughly 100 mL 20X SSC Solution with 850 mL purified water. Add 1 mL NP-40 and mix thoroughly until NP-40 is completely dissolved. Measure pH at room temperature using a pH meter and adjust to pH  $7.0 \pm 0.2$  with 1 N NaOH. Add purified water to bring final volume to 1 L. Mix and filter through a 0.45 µm filtration unit. Store prepared stock solution at room temperature. Discard stock solution after 6 months or sooner if solution appears cloudy or contaminated. Discard solution that was used in the assay at the end of each day.

### Specimen Collection, Processing, Storage, and Slide Preparation

#### Specimen Collection and Processing

Bone marrow collection should be performed according to the laboratory's institution guidelines. The *AGT Cytogenetics Laboratory Manual* contains recommendations for specimen collection, culturing, and harvesting. It states that it is acceptable to collect bone marrow in either sodium-heparinized transport medium or a sodium-heparinized vacutainer.<sup>15</sup> The manual also recommends that the specimens be transported to the cytogenetics laboratory, and cultures of the cells should be initiated immediately. Bone marrow should not be frozen prior to culturing or harvesting. Harvested bone marrow cells can be used immediately to prepare slides, or stored in fixative at  $-20^{\circ}\text{C}$ .

Hemolyzed specimens may prevent proper culture for standard cytogenetic analysis. Exposure of the specimens to acids, strong bases, or extreme heat should be avoided. Such conditions are known to damage DNA and may result in FISH assay failure.

### Slide Preparation from Fixed Cell Pellets

For the slides presented in the **SPECIFIC PERFORMANCE**

**CHARACTERISTICS** section of this document, the following method for slide preparation was used:

1. Centrifuge the fixed cell pellet(s) at 1100 rpm for 8 minutes at room temperature ( $15$  to  $30^{\circ}\text{C}$ ).
  2. Remove the supernatant to within approximately 0.5 to 1.0 mL of the cell pellet, being careful not to disturb the pellet.
  3. Resuspend the pellet in the remaining 0.5 to 1.0 mL of supernatant by gently agitating the tube.
  4. For optimal chromosome spreading results, a cytogenetic drying chamber may be used. Prewarm the unit to a temperature of  $28 \pm 2^{\circ}\text{C}$  with a relative humidity of  $35 \pm 2\%$ . If a cytogenetic drying chamber is not available, a fume hood may be used as an alternative.
  5. Ensure the sample is mixed adequately before preparing the slide. Using a transfer pipette, expel 3 drops of cell suspension along the length of a precleaned, wet slide (that has been stored in cold water) while holding the slide at an approximately  $45^{\circ}$  angle and blotting the bottom edge on a paper towel.
  6. Allow the slide to completely dry before removing from the drying chamber or fume hood.
  7. Label each slide appropriately.
  8. Using a phase contrast microscope, examine the number of interphase nuclei per field, under low power (10X objective). A minimum of 100 cells per low power field is suggested for optimum assay results. Adjust the cell specimen suspension with fresh Carnoy's fixative to achieve the recommended number of interphase nuclei.
- NOTE: An optimal specimen will contain little to no debris and/or cytoplasm.**
9. Once optimal cell suspension is obtained and slides have been prepared, add 2 to 5 mL of fresh 3:1 methanol/acetic acid fixative to the remaining cell pellet before storage.
  10. Age slides using a slide warmer at  $56 \pm 2^{\circ}\text{C}$  for 30 minutes or at room temperature overnight.

#### Slide and Fixed Pellet Storage

Place the prepared slides in a covered slide box. Seal the slide box in a plastic bag containing approximately 1 tablespoon of desiccant. Store at  $-20^{\circ}\text{C}$  ( $\pm 10^{\circ}\text{C}$ ) prior to hybridization or proceed to **Specimen Target Preparation**.

**NOTE: Fixed slides are stable at  $-20^{\circ}\text{C}$  ( $\pm 10^{\circ}\text{C}$ ) for up to 12 months. Store any remaining fixed pellets at  $-20^{\circ}\text{C}$  ( $\pm 10^{\circ}\text{C}$ ) for up to 1 month in the event preparation of additional slides is necessary.**

### Specimen Target Preparation

**NOTE: Initiate Automated Probe Denaturation/Hybridization procedure prior to completing Step 4 of this section to ensure the materials have adequate time to thaw.**

1. Transfer 2X SSC Solution to a Coplin jar.
  2. Transfer 2X SSC Coplin jar to a hot water bath for approximately 30 minutes prior to using in order to ensure the solution reaches a temperature of  $37 \pm 1^{\circ}\text{C}$ .
  3. Verify the temperature of the 2X SSC Solution is  $37 \pm 1^{\circ}\text{C}$  using a calibrated thermometer.
  4. Immerse the previously aged specimen slides in 2X SSC Solution for 30 minutes at  $37 \pm 1^{\circ}\text{C}$ .
- NOTE: Immerse no more than 4 slides simultaneously in each Coplin jar.**
5. Using forceps, remove specimen slides from the 2X SSC Solution, immediately transfer the specimen slides to Coplin jars containing 70% EtOH for a minimum of 2 minutes, and agitate the specimen slides within Coplin jar for 1 to 3 seconds. Following 70% EtOH, transfer to 85% EtOH for a minimum of 2 minutes, and then to 100% EtOH for a minimum of 2 minutes.
  6. Allow slides to air dry.
  7. Following dehydration in EtOH, the slide may be placed on a  $56 \pm 2^{\circ}\text{C}$  slide warmer for up to 2 minutes to ensure complete drying prior to application of the probe.

**NOTE: Keep the specimen slides in 100% EtOH until you are ready to dry all slides and apply the probe mixture.**

### Automated Probe Denaturation/Hybridization

1. Remove the DNA probe(s), Vysis LSI/WCP Hybridization Buffer, and purified water from storage and allow the reagents to reach room temperature.



- Vortex DNA probe(s) and Vysis LSI/WCP Hybridization Buffer for 2 to 3 seconds.
- Centrifuge tubes for 2 to 3 seconds.
- Transfer 7  $\mu$ L Vysis LSI/WCP Hybridization Buffer, 2  $\mu$ L purified water, and 1  $\mu$ L DNA probe into a 1.5 mL microcentrifuge tube.
- Vortex and centrifuge the mixture again briefly.
- Using a microliter pipettor, apply 10  $\mu$ L of probe mixture to the specimen target and immediately apply coverslip without introducing bubbles.
- Seal coverslips using a syringe filled with rubber cement.
- Prior to adding specimen slides, insert two ThermoBrite humidity cards into the slot positions of the ThermoBrite unit lid. Allow the tabs within the lid to support the cards. Refer to the ThermoBrite Operator's Manual for instructions regarding the reuse of humidity cards in subsequent runs.
- After ThermoBrite humidity cards are inserted, saturate the strips with distilled water. Apply 8 to 10 mL of distilled or deionized water to each card for the first operation.
- Switch the power to ThermoBrite Denaturation/Hybridization System to ON.

**NOTE: The ThermoBrite main power switch is located on the rear panel. The instrument will beep to announce power has been turned ON. The main Menu will be displayed when the instrument has reached 37°C.**

- Set the ThermoBrite program for the following parameters:
  - Denat Time: 2 minutes
  - Denat Temp: 73°C
  - Hyb Time: 12 to 18 hours
  - Hyb Temperature: 37°C
  - Denat = Denaturation
  - Hyb = Hybridization
- When prompted, place specimen slides on the heating surface of the ThermoBrite. Gently push the specimen slides toward the middle of the plate, butting the edge of the slide into the marked positions in the slide locator. The frosted edge of the slide should hang over the edge of the heating surface. Confirm the slides lay flat and are properly aligned into the marked positions in the slide locator.
 

**NOTE: If hybridizing less than 12 slides, use blank slides to bring the total slide number to 12.**
- Close the ThermoBrite lid. The cursor should highlight the "Run a PGM" line. Press the "Enter" button to accept.

### Wash Procedure

- Transfer 70 mL of 0.4X SSC/0.3% NP-40 and 70 mL of 2X SSC/0.1% NP-40 into individual Coplin jars. Transfer Coplin jar containing 0.4X SSC/0.3% NP-40 to a hot water bath at least 60 minutes prior to use. Use 2X SSC/0.1% NP-40 at room temperature. Use solutions for 1 day only, and then discard.
- Verify the temperature of the 0.4X SSC/0.3% NP-40 solution is 73  $\pm$  1°C using a calibrated thermometer.
- Remove specimen slides from the ThermoBrite.
- Remove coverslips from one slide and immediately immerse the slide in the 0.4X SSC/0.3% NP-40. Agitate the specimen slides within Coplin jar for 1 to 3 seconds. Repeat process with other slides up to a total of four slides. Start timing when the fourth slide is immersed.
 

**NOTE: To maintain the proper temperature in 0.4X SSC/0.3% NP-40, wash 4 slides simultaneously. If you have less than 4 slides, add blank slides that are at room temperature to bring the total to 4.**
- Remove slides after 3.5 minutes.
- Immerse slides in Coplin jar containing 2X SSC/0.1% NP-40. Agitate the specimen slides within Coplin jar for 3 to 5 seconds.
- Remove slides from 2X SSC/0.1% NP-40 after slides have been immersed for 60 seconds.
 

**NOTE: If washing additional slides, ensure the temperature of the wash solution is 73  $\pm$  1°C before washing.**

### Counterstain Procedure

- Dry each slide by touching the bottom edge to a paper towel (or equivalent) and wiping the underside (the side that does not contain specimen).
- Air-dry the slides in darkness on long edge of the slide to facilitate evaporation and to prevent pooling of 2X SSC/0.1% NP-40 for a maximum of 2 hours.
- Remove DAPI II Counterstain from storage and allow the reagent to reach room temperature.

- Vortex DAPI II Counterstain for 2 to 3 seconds.
- Centrifuge tube for 2 to 3 seconds.
- Using a microliter pipettor, apply 10  $\mu$ L of DAPI II Counterstain to each specimen target area on the slide and apply coverslips. Repeat for each slide.
- Allow a minimum of 10 minutes prior to microscopic observation.

### Archiving Procedure

Store hybridized slides at -20°C ( $\pm$  10°C) while protecting from light. Under these conditions, the slides can be stored for up to 3 weeks after the application of DAPI II Counterstain without significant loss in fluorescence signal intensity.

### Slide Examination

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...	Allow simultaneous excitation and emission of ....
Single Band Green	SpectrumGreen fluorophore
Single Band Orange	SpectrumOrange fluorophore
Dual Band Green/Orange V2	SpectrumOrange and SpectrumGreen fluorophores
Triple Band – DAPI, Green and Orange	DAPI, SpectrumOrange and SpectrumGreen fluorophores

Routine reading is conducted using a Triple Band or Dual Band filter. For an individual nucleus, if specific probe signal(s) appear weak with a Triple Band or Dual Band filter, it is recommended to use a Single Band Green or Single Band Orange filter to assist in enumeration.

### Interpretation and Result Reporting

#### Quality Control

##### Assessing Slide Adequacy

Evaluate slide hybridization adequacy using the following criteria. If the criteria are not met, the specimen slide should not be evaluated.

- Nuclear Morphology: Borders of cell nuclei should generally be distinguishable and be intact.
- Background: The background should appear dark or black and be relatively free of fluorescent particles or haziness.
- Probe Signal Intensity: The signals should be bright, compact, round or oval shapes, distinct, and easily evaluable.

#### Signal Enumeration

Using the appropriate filters listed above, two technologists each score 100 nuclei for each hybridization target by counting and recording the number of orange and green signals present in each nucleus. The first reader scores the nuclei on the left side of the hybridization target (as best determined) and the second reader scores the nuclei on the right side of the hybridization target (as best determined). Refer to Table 1 for Dual Color Signal Counting Guide.

- Select only intact nuclei that are not folded, overlapped, or obstructed by debris.
- Avoid scoring slides or areas within slides that have excessive nonspecific hybridization or many nuclei that have too few or no signals.
- Avoid scoring nuclei with clumps or clouds of signals.
- Signals of the same color that are touching, regardless of the size, are counted as one signal. If there is a small strand of signal connecting separated signals, also count as one signal.
- If a signal area has no gaps greater than a signal width for an intact signal for that same probe, count as one signal. If an intact signal size for that same probe could be placed between separated signals of that same color and no "connecting" signal is present, consider the separated signal to be an additional signal.
- If a specimen has a low level abnormal FISH pattern, use of the appropriate single-pass filter to confirm the pattern is recommended. Failure to follow this recommendation may result in inaccurate identification of signals.
- Signals of the same color may not be the same intensity in a given nucleus. Therefore, it may be necessary to use the appropriate single-pass filter and/or adjust the focal plane.
- If there is any doubt as to whether or not a cell should be scored, do not score the cell.

**Table 1. Dual Color Signal Counting Guide**

Key: ○ = Green  
● = Orange

1		If nuclei are overlapping, do not count.
2		Count as two orange signals and two green signals. One orange signal is diffuse.
3		Count as two orange signals and two green signals. One orange signal is split.
4		Count as one orange signal and two green signals. One green signal is split and the orange signal is split.
5		Count as two orange signals and one green signal.

After recording the results, the number of 1R2G (1 orange, 2 green) abnormal signal pattern(s) from each reader are examined and the following stepwise rules are applied to determine the final 200 nuclei count. Refer to Table 2 for Rules for Combining Scores and Use of Third Reader. For a detailed explanation of the rules in Table 2, see the text following the table.

**Table 2. Rules for Combining Scores and Use of Third Reader**

Number	Rule	Yes	No
1	Is one count at or below 6 and the other count above 6?	Go to 6.	Go to 2.
2	Do the two counts differ by 5 or less?	Go to 5.	Go to 3.
3	Is either of the two counts 12 or less?	Go to 6.	Go to 4.
4	Do the two counts differ by more than 15?	Go to 6.	Go to 5.
5	Combine the two counts for final value.		
6	The third technologist determines the 1R2G count per 100 scoreable nuclei. The two closest of the three counts are combined for the final 200 nuclei count. If all three counts are equidistant, the final count is double the median.		

- If one reader has an 1R2G count at or below 6 and the other reader has an 1R2G count above 6, a third reader will score 100 nuclei within the hybridization target. Of the three scores, the two scores closest to one another will be combined to generate the count for 200 nuclei. If all three scores are equidistant, the median value is doubled and used as the score.
  - If the 1R2G counts determined by the two readers differ by 5 or less per 100 nuclei evaluated, then the two scores are added together to generate the count for 200 nuclei per specimen.
  - If the 1R2G counts determined by the two readers differ by more than 5 per 100 nuclei evaluated, then a decision is made based on the proximity of the number of 1R2G counts to 12.
- If either of the two readers have 1R2G counts of 12 or less and if the two counts differ by more than 5 per 100 nuclei evaluated, then a third reader will score 100 nuclei within the hybridization target. Of the three scores, the two scores closest to one another will be combined to generate the count for 200 nuclei. If all three scores are equidistant, the median value is doubled and used as the score.
  - If each of the two readers' 1R2G counts are greater than 12 and if those counts differ by 15 or fewer per 100 nuclei, the scores will be combined to generate the count for 200 nuclei. If the two readers have 1R2G counts which differ by more than 15 per 100 nuclei evaluated, then a third reader will score 100 nuclei within the hybridization target. Of the three scores, the two scores closest to one another will be combined to generate the count for 200 nuclei. If all three scores are equidistant, the median value is doubled and used as the score.

### Interpretation of Results

#### Normal/Abnormal Determination

Normal/Abnormal determination for LSI EGR1 is made for each specimen by comparing the number of observed 1R2G patterns per 200 scoreable nuclei to the normal cut-off value (Table 3). If the number of 1R2G nuclei is greater than 12, the result is abnormal. If the number of 1R2G nuclei is 12 or less, the result is normal.

**Table 3. Normal Cut-off Value for Determination of Normal/Abnormal**

Probe/Color	Abnormal Pattern	Cut-Off: Number of Patterns per 200 Nuclei	Abnormality
LSI EGR1/orange (R) D5S23, D5S721/green (G)	1R2G	>12	5q–

One orange and two green signals are expected in a cell with loss of one copy of the LSI EGR1 probe target and retention of both copies of the LSI D5S23, D5S721 region.

### LIMITATIONS OF THE PROCEDURE

- FOR IN VITRO DIAGNOSTIC USE ONLY.
- The Vysis EGR1 FISH Probe Kit is intended to be used in combination with additional biomarkers, morphology, and other clinical information.
- If a specimen has a low level abnormal FISH pattern, use of the appropriate single-pass filter to confirm the pattern is recommended. Failure to follow this recommendation may result in inaccurate identification of signals.
- Other abnormal signal patterns may occur, and metaphase analysis may be helpful in characterization of such patterns.

### SPECIFIC PERFORMANCE CHARACTERISTICS

#### Analytical Specificity

Analytical specificity is defined as the percentage of signals that hybridize to the correct locus and no other location. The analytical specificity of the Vysis LSI EGR1 D5S23, D5S721 probes for their respective chromosome target loci was established using metaphase chromosomes prepared from peripheral blood cultures of five karyotypically normal males that were pooled prior to dropping on microscope slides. The hybridization location of each FISH signal on chromosomes of 100 consecutive metaphase nuclei was evaluated by one technologist for a total of 200 target loci.

For each probe and sample, the number of metaphase chromosome FISH signals hybridized to the correct locus and the number of metaphase chromosome FISH signals hybridized to the incorrect locus were enumerated. The analytical specificity of each probe was calculated as the number of metaphase chromosome FISH signals hybridized to the correct locus divided by the total number of metaphase chromosome FISH signals hybridized and multiplied by 100 to give a percentage.

The analytical specificity of the Vysis LSI EGR1 SpectrumOrange/ D5S23, D5S721 SpectrumGreen Probes was 100%, as shown in Table 4.

**Table 4. Analytical Specificity**

Probe	No. of Metaphase Chromosome Signals		Analytical Specificity (%)	95% Confidence Interval
	Hybridized to the Correct Target Locus	Total Hybridized Signals		
D5S23, D5S721	200	200	100	(98, 100)
EGR1	200	200	100	(98, 100)

#### Analytical Sensitivity

Analytical sensitivity is defined as the percentage of scoreable interphase nuclei with the expected normal signal pattern. The expected normal interphase signal pattern for the probes in the Vysis EGR1 FISH Probe Kit is 2R2G per nucleus.

The analytical sensitivity of the Vysis LSI EGR1 SpectrumOrange/ D5S23, D5S721 SpectrumGreen Probes was established using interphase nuclei prepared from 25 bone marrow specimens that were either karyotypically normal or 5p15 and 5q31 deletion-free. The orange and green signal patterns of nuclei for 25 specimens were evaluated by two technologists. Each technologist evaluated 100 nuclei per specimen for a total of 200 nuclei per specimen and 5000 scoreable nuclei from normal specimens.

The analytical sensitivity was calculated as the percentage of scoreable interphase nuclei with the expected 2R2G signal pattern at the 95% confidence interval.

The Vysis EGR1 FISH Probe Kit has an analytical sensitivity of 99.6%, as shown in Table 5.

**Table 5. Analytical Sensitivity**

Probe Kit	No. of Interphase Chromosome Signals		Analytical Sensitivity	
	With Expected Signal Pattern	Scoreable Signals	Point Estimate	95% Confidence Interval
LSI EGR1/D5S23, D5S721	4979	5000	99.6	(99.4, 99.7)

**Verification of Normal Cut-off**

The normal cut-off value is defined as the maximum quantity of scoreable interphase nuclei with a specific abnormal signal pattern at which a specimen is considered normal for that signal pattern. The normal cut-off value is expressed in terms of a percentage or the actual number of a specific abnormal nuclear FISH signal pattern per the standard number of nuclei tested.

The normal cut-off value for this assay is 6% or 12 1R2G patterns per 200 scoreable interphase nuclei. Specimens exceeding 12 1R2G patterns per 200 scoreable nuclei are considered abnormal for deletion of the Vysis LSI EGR1 probe target. This 6% normal cut-off value was adopted from the publication of Vance et al, who utilized the Vysis LSI ERG1/D5S23, D5S721 probe set in a study that established a high level of agreement between cytogenetics and FISH in 237 blood and bone marrow specimens studied at AML diagnosis.<sup>3</sup>

In order to confirm that the 6% normal cut-off served well to prevent normal specimens from being called abnormal, the assay was performed on interphase nuclei from 25 bone marrow specimens from either karyotypically normal specimens or 5p15.2 and 5q31 deletion-free specimens. The signal patterns of 200 nuclei were evaluated by counting the number of orange and green signals. Each of two technologists evaluated 100 nuclei per specimen. Among the 25 normal specimens, none produced 1R2G signals at or above the 6% normal cut-off.

**Reproducibility**

Two replicates of the assay were run on 2 high-positive, 2 low-positive, and 2 normal specimens at three sites on 5 different days. The positive specimens for the site-to-site study were obtained by mixing positive bone marrow cells with normal bone marrow cells to obtain the desired levels of abnormality. The mean and the standard deviations of the percentage of cells with the 1R2G signal pattern was calculated. Results shown in Table 6 show the overall agreement with the normal/abnormal status of the test specimens. All sites obtained 100% agreement with the known status of all 6 specimens on all 5 days, except one site which had one discordant result for a normal specimen.

**Table 6. Overall Agreement, Site to Site**

Category	Number			Percent Agreement
	Agree	Disagree	Total	
High Positive	60	0	60	100
Low Positive	60	0	60	100
Normal	59	1	60	98

The analysis of variance components for the site-to-site study is shown in Table 7.

**Table 7. Site-to-Site Analysis of Variance Components**

Sample	N	Mean <sup>a</sup>	Within Day SD <sup>b</sup>	Between Day SD	Between Site SD	Total SD
High Positive 1	30	70.0	3.28	4.01	5.44	7.51
High Positive 2	30	47.6	5.56	0.00	0.74	5.61
Low Positive 1	30	18.1	3.00	3.82	1.03	4.97
Low Positive 2	30	14.9	3.25	1.54	0.00	3.59
Normal 1	30	0.7	0.71	0.00	0.68	0.99
Normal 2	30	0.9	0.66	1.42	0.22	1.59

<sup>a</sup> Percentage of cells with 1R2G signal patterns  
<sup>b</sup> SD = standard deviation

Using the same specimens from the site-to-site study, four replicates of the assay were run on 2 high-positive, 2 low-positive, and 2 normal specimens using 3 different lots of probe at a single site. The overall agreement with the known normal/abnormal status of the test specimen is shown in Table 8. All replicates using the three probe lots for each of the 6 specimens produced agreement with the known status of the specimens.

**Table 8. Overall Agreement, Lot to Lot**

Category	Number			Percent Agreement
	Agree	Disagree	Total	
High Positive	24	0	24	100
Low Positive	24	0	24	100
Normal	24	0	24	100

The analysis of variance components for the lot-to-lot study is shown in Table 9.

**Table 9. Lot-to-Lot Analysis of Variance Components**

Sample	N	Mean <sup>a</sup>	Within Lot SD <sup>b</sup>	Between Lot SD	Total SD
High Positive 1	12	66.2	7.19	0.00	7.19
High Positive 2	12	47.4	3.69	3.04	4.78
Low Positive 1	12	12.7	4.29	0.00	4.29
Low Positive 2	12	12.3	1.84	1.12	2.15
Normal 1	12	0.0	0.00	0.00	0.00
Normal 2	12	0.1	0.14	0.20	0.25

<sup>a</sup> Percentage of cells with 1R2G signal patterns

<sup>b</sup> SD = standard deviation

In these reproducibility studies, 84 assays were run on low-positive specimens. None of the 84 low-positive assays resulted in a 1R2G signal pattern at or below 6%.

**Clinical Utility**

The clinical utility of the cytogenetic detection of the deletion of chromosome 5q is correlated with reduced 5-year overall survival in studies by Byrd et al<sup>7</sup> and Grimwade et al.<sup>8</sup> The Byrd publication demonstrated the prognostic value in 86 patients with a -5/5q- abnormality by a median overall survival (OS) of 0.3 years compared to the median OS of 1.3 years associated with patients exhibiting a normal karyotype. Patients with a -5/5q- abnormality had a 5-year OS of 6% compared to a 5-year OS of 24% associated with patients exhibiting a normal karyotype. Patients with a -5/5q- abnormality also had a significantly lower complete remission (CR) rate of 31% than the normal karyotype, which resulted in a CR of 68% with a  $p < 0.001$ . For del(5q), 42 patients had a 5-year OS of 5%, (95% CI 2 – 13%) and median overall survival of 0.3 years.

Grimwade et al, showed prognostic value in 28 patients for 5q-. Cytogenetic abnormalities in the Medical Research Council (MRC) AML 10 clinical trial for del(5q) patients showed 5-year OS of 11% and CR of 57% in the adverse risk group. These values varied significantly from the no abnormality, or normal karyotype group, which had 5-year OS of 42% and CR of 88% ( $p < 0.001$ ).

The publication "Utility of interphase FISH to stratify patients into cytogenetic risk categories at diagnosis of AML in an Eastern Cooperative Oncology Group (ECOG) clinical trial (E1900)" by Vance et al,<sup>3</sup> establishes linkage between cytogenetic results and the Vysis EGR1 FISH Probe kit. When 181 bone marrow specimens were compared to cytogenetic results at the > 6% cut-off, there was overall agreement of 98.90% (179/181) (95% CI 96.06% – 99.70%), negative percent agreement of 100% (171/171) (95% CI 97.80% – 100.00%) and positive percent agreement of 80% (8/10) (95% CI 49.02% – 94.33%). Results are presented below:

		Karyotype –5/del5q		
		Positive	Negative	Total
FISH (1R2G –5q deletion signal pattern)	Positive	8	0	8
	Negative	2 <sup>a,b</sup>	171	173
	Total	10	171	181

<sup>a</sup> Cytogenetic result was –5/del(5q). FISH signal pattern was 44% 1R1G (monosomy of chromosome 5).

<sup>b</sup> Cytogenetic result was –5/del(5q). FISH signal pattern was 1% 1R2G. False negative results.

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## TECHNICAL ASSISTANCE

For technical assistance, call Abbott Molecular Technical Services at +1-800-533-7042 in the US and from outside the US +49-6122-580 or visit the Abbott Molecular Worldwide Web site at <http://www.abbottmolecular.com>.


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