Vysis EGR1 FISH Probe Kit– SC (Specimen Characterization)

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REF 04N37-001

30-608500/R2

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Key to Symbols Used

REF	Reference Number
LOT	Lot Number
IVD	In Vitro Diagnostic Medical Device
GTIN	Global Trade Item Number
	Use by
	Temperature Limit
	Refer to **WARNINGS AND PRECAUTIONS** section
	Refer to **WARNINGS AND PRECAUTIONS** section
	Refer to **WARNINGS AND PRECAUTIONS** section
	Refer to **WARNINGS AND PRECAUTIONS** section
	Biological Risks
	Caution, consult accompanying documents
	Consult instructions for use
Σ	Contains sufficient for <n> tests
	Manufacturer
EC REP	Authorized Representative in the European Community

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NAME

Vysis EGR1 FISH Probe Kit – SC (Specimen Characterization) containing the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes

INTENDED USE

The Vysis EGR1 FISH Probe Kit – SC (Specimen Characterization) detects the LSI EGR1 probe target on chromosome 5q in bone marrow specimens. The Vysis EGR1 FISH Probe Kit – SC assay results characterize bone marrow specimens from patients with acute myeloid leukemia or myelodysplastic syndrome. The assay results are intended to be interpreted by a qualified pathologist or cytogeneticist.

This device is not intended for high-risk uses such as selecting therapy, predicting therapeutic response or disease screening. The use of this product for diagnosis, monitoring or risk assessment has not been established.

SUMMARY AND EXPLANATION OF THE TEST

Abnormalities of chromosome 5 are common aberrations in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML).[1] 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.[2] A study suggests that haploinsufficiency of EGR1 may play a role in leukemogenesis.[3] The Vysis locus-specific identifier (LSI) EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes, components of the Vysis EGR1 FISH Probe Kit – SC, have been used in several studies to detect EGR1 deletions.[4-8] A study comparing the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes to metaphase cytogenetics to detect loss of 5q, in both MDS and AML, concluded while cytogenetics detected most instances of del (5q), fluorescence in situ hybridization (FISH) was especially useful in cases with suboptimal growth, and EGR1 FISH detects del(5q) in a broad variety of myeloid neoplasms.[8] A study investigating whether monosomy 5, identified by G-banded karyotyping, truly existed in 28 cases of MDS or AML, found del(5q) in 24 cases and monosomy 5 in only 3 cases. This study concluded EGR1 FISH, using the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes, can complement conventional cytogenetics and improve the karyotype definition.[1] A study, conducted as part of an Eastern Cooperative Oncology Group (ECOG) clinical trial, showed that FISH detection of specific aberrations using several Vysis FISH probe sets including the Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes, was highly correlated to cytogenetic discovery of these same aberrations in AML patients.[4]

The Vysis EGR1 FISH Probe Kit – SC uses FISH DNA probe technology to detect the probe target for LSI EGR1, and the LSI D5S23, D5S721 probe serves as a control.

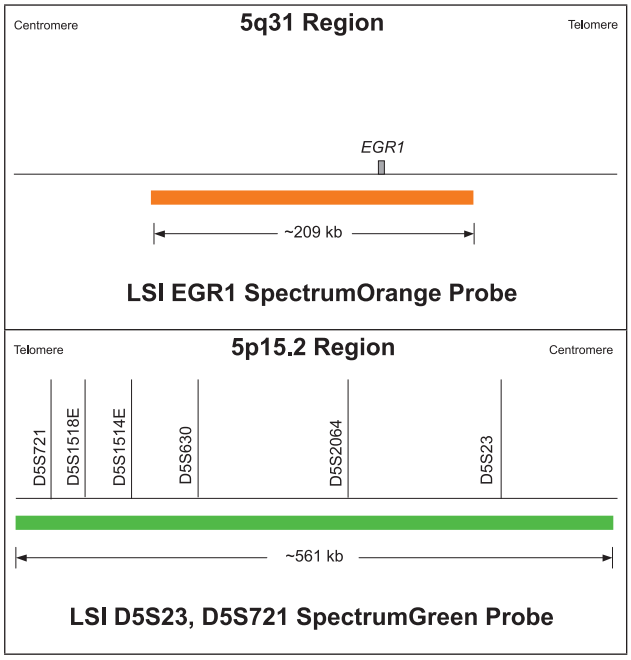
BIOLOGICAL PRINCIPLES OF THE PROCEDURE

DNA Probe Description

Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes

The SpectrumOrange-labeled LSI EGR1 probe, approximately 209 kb in length (chr5:137654208-137862738; February 2009 Assembly; UCSC Human Genome Browser[9]), is located at 5q31 and contains the complete EGR1 gene.

The SpectrumGreen-labeled LSI D5S23, D5S721 probe, approximately 561 kb in length (chr5:9397109-9958407; February 2009 Assembly; UCSC Human Genome Browser[9]), is located at 5p15.2.



General 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 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 using appropriate controls[10] and analytical techniques as well as taking into consideration other clinical and diagnostic test data.

Bone marrow cells 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 typical 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, 1 SpectrumOrange signal (LSI EGR1) and 2 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 atypical signal patterns of interest.

REAGENTS

Materials Provided

This kit contains 5 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.	30-171027
Quantity	1 vial, 20 µL per vial (100 and 300 ng/µL)
Storage	–20°C (±5°C) and protected from light
Composition	SpectrumOrange and SpectrumGreen fluorophore-labeled DNA probes

Vysis LSI/WCP Hybridization Buffer

Part No.	30-804813
Quantity	1 vial, 150 µL per vial
Storage	–20°C (±5°C)
Composition	Dextran sulfate, formamide, and SSC

DAPI II Counterstain

Part No.	30-804811
Quantity	1 vial, 600 µL per vial (125 ng/mL)
Storage	–20°C (±5°C) and protected from light
Composition	DAPI (4′,6-diamidino-2-phenylindole•2HCl) in phenylenediamine dihydrochloride, glycerol, and buffer

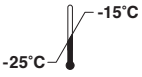
NP-40

Part No.	30-804810
Quantity	2 vials, 2000 µL per vial
Storage	–25°C to 30°C
Composition	NP-40 (non-ionic detergent)

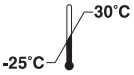
20X SSC Salt

Part No.	30-804812
Quantity	1 bottle, 66 g
Storage	–25°C to 30°C
Composition	Sodium chloride and sodium citrate

REAGENT STORAGE AND HANDLING INSTRUCTIONS



The Vysis EGR1 FISH Probe Kit – SC must be stored at –20°C (±5°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

Warning:

- The assay results are intended to be interpreted only by a qualified pathologist or cytogeneticist.
- The Vysis EGR1 FISH Probe Kit – SC is not for high-risk uses such as selecting therapy, predicting therapeutic response or disease screening.
- The use of this product for diagnosis, monitoring or risk assessment has not been established.

CAUTION: Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which he practices, to use or order the use of the device.

Safety Precautions

Vysis LSI EGR1 SpectrumOrange/D5S23, D5S721 SpectrumGreen 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,[11] OSHA Standards on Bloodborne Pathogens,[12] CLSI Document M29-A3,[13] and other appropriate biosafety practices.[14] 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.[11]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[14]

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.

NP-40

Danger

Hazard-determining components of labeling: Polyethylene glycol octylphenyl ether

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.
P310	Immediately call a POISON CENTER or doctor/physician.
P301+P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P302+P352	IF ON SKIN: Wash with plenty of water.
P332+P313	If skin irritation occurs: Get medical advice/attention.
P330	Rinse mouth.
P362	Take off contaminated clothing and wash before reuse.
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.

Handling Precautions

- The Vysis EGR1 FISH Probe Kit – SC 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 – SC 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 photobleached 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 – SC 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 – SC, verification of the upper reference limit is recommended.

ASSAY PROCEDURE

Materials Provided

- Vysis EGR1 FISH Probe Kit – SC (List No. 04N37-001)

Materials Required But Not Provided

Laboratory Reagents

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

Laboratory Equipment

- Glass microscope slides, precleaned
- 22 mm × 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)
- Forceps
- Disposable syringe (5 mL)
- Disposable pipettes (5 to 20 mL)
- Coplin jars/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
- 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 ≥ 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 60/63X 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 – SC 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, or equivalents.

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. Discard stock solution after 6 months or sooner if solution appears cloudy or contaminated. Dilutions in Coplin jars may be used for up to 1 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 ± 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 ± 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 blood collection tube, such as BD Vacutainer® Blood Collection Tube.[15] The manual also recommends that the specimens be transported to the cytogenetics laboratory and cultures 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˚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˚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 ± 2˚C with a relative humidity of 35 ± 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˚ 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 ± 2˚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˚C (±10˚C) prior to hybridization or proceed to **Specimen Target Preparation**.

NOTE: Fixed slides are stable at −20˚C (±10˚C) for up to 12 months. Store any remaining fixed pellets at −20˚C (±10˚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±1˚C.
3. Verify the temperature of the 2X SSC Solution is 37±1˚C using a calibrated thermometer.
4. Immerse the previously aged specimen slides in 2X SSC Solution for 30 minutes at 37±1˚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% ethanol for a minimum of 2 minutes, and agitate the specimen slides within Coplin jar for 1 to 3 seconds. Following 70% ethanol, transfer to 85% ethanol for a minimum of 2 minutes, and then to 100% ethanol for a minimum of 2 minutes.
6. Allow slides to air-dry.
7. Following dehydration in ethanol, the slide may be placed on a 56±2˚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% ethanol until you are ready to dry all slides and apply the probe mixture.

Automated Probe Denaturation/Hybridization

1. 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.
2. 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
3. Remove the DNA probe(s), Vysis LSI/WCP Hybridization Buffer, and purified water from storage and allow the reagents to reach room temperature.
4. Vortex DNA probe(s) and Vysis LSI/WCP Hybridization Buffer for 2 to 3 seconds.
5. Centrifuge tubes for 2 to 3 seconds.
6. Transfer 7 µL Vysis LSI/WCP Hybridization Buffer, 2 µL purified water, and 1 µL DNA probe into a 1.5 mL microcentrifuge tube.
7. Vortex and centrifuge the mixture again briefly.
8. Using a microliter pipettor, apply 10 µL of probe mixture to the specimen target and immediately apply coverslip without introducing bubbles.
9. Seal coverslips using a syringe filled with rubber cement.
10. Prior to adding specimen slides, insert 2 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.
11. 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.
12. 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.
13. Close the ThemoBrite lid. The cursor should highlight the "Run a PGM" line. Press the "Enter" button to accept.

Wash Procedure

1. Transfer a sufficient volume of 0.4X SSC/0.3% NP-40 and 2X SSC/0.1% NP-40 into individual Coplin jars to cover the slides. Transfer Coplin jar containing 0.4X SSC/0.3% NP-40 to a water bath. Heat until the internal temperature of the 0.4X SSC/0.3% NP-40 solution is 73±1˚C. Confirm the solution temperature using a calibrated thermometer. Use 2X SSC/0.1% NP-40 at room temperature. Use solutions for 1 day only, and then discard.
2. Remove specimen slides from the ThermoBrite.
3. Remove coverslip from 1 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 4 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.
4. Remove slides after 3.5 minutes.
5. Immerse slides in Coplin jar containing 2X SSC/0.1% NP-40. Agitate the specimen slides within Coplin jar for 3 to 5 seconds.
6. Remove slides from 2X SSC/0.1% NP-40 after slides have been immersed for 5 to 60 seconds.
   NOTE: If washing additional slides, ensure the temperature of the wash solution is 73±1˚C before washing.

Counterstain Procedure

1. 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).
2. 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.
3. Remove DAPI II Counterstain from storage and allow the reagent to reach room temperature.
4. Vortex DAPI II Counterstain for 2 to 3 seconds.
5. Centrifuge tube for 2 to 3 seconds.
6. Using a microliter pipettor, apply 10 µL of DAPI II Counterstain to each specimen target area on the slide and apply coverslips. Repeat for each slide.
7. Allow a minimum of 10 minutes prior to microscopic observation.

Archiving Procedure

Store hybridized slides at −20˚C (±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 (or equivalent)…	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.

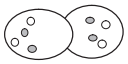
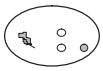
Signal Enumeration

The signal enumeration instructions are based upon a 6% upper reference limit adopted from Vance et al.[4]

Using the appropriate filters listed above, 2 technologists (readers) 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 1 signal. If there is a small strand of signal connecting separated signals, also count as 1 signal.
- If a signal area has no gaps greater than a signal width for an intact signal for that same probe, count as 1 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 an overall low signal intensity FISH pattern and a low level of cells with an atypical (1R2G) FISH signal 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 2 orange signals and 2 green signals. One orange signal is diffuse.
3		Count as 2 orange signals and 2 green signals. One orange signal is split.
4		Count as 1 orange signal and 2 green signals. One green signal is split and the orange signal is split.
5		Count as 2 orange signals and 1 green signal.

After recording the results, the number of 1R2G (1 orange, 2 green) atypical FISH 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** which is based on an upper reference limit of 6%. 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 1 count at or below 6 and the other count above 6?	Go to 6.	Go to 2.
2	Do the 2 counts differ by 5 or less?	Go to 5.	Go to 3.
3	Is either of the 2 counts 12 or less?	Go to 6.	Go to 4.
4	Do the 2 counts differ by more than 15?	Go to 6.	Go to 5.
5	Combine the 2 counts for final value.		
6	The third reader determines the 1R2G count per 100 scoreable nuclei. The 2 closest of the 3 counts are combined for the final 200 nuclei count. If all 3 counts are equidistant, the final count is double the median.		

- If 1 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 3 scores, the 2 scores closest to one another will be combined to generate the count for 200 nuclei. If all 3 scores are equidistant, the median value is doubled and used as the score.
  - If the 1R2G counts determined by the 2 readers differ by 5 or less per 100 nuclei evaluated, then the 2 scores are added together to generate the count for 200 nuclei per specimen.
  - If the 1R2G counts determined by the 2 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 2 readers have 1R2G counts of 12 or less and if the 2 counts differ by more than 5 per 100 nuclei evaluated, then a third reader will score 100 nuclei within the hybridization target. Of the 3 scores, the 2 scores closest to one another will be combined to generate the count for 200 nuclei. If all 3 scores are equidistant, the median value is doubled and used as the score.
  - If each of the 2 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 2 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 3 scores, the 2 scores closest to one another will be combined to generate the count for 200 nuclei. If all 3 scores are equidistant, the median value is doubled and used as the score.

Interpretation of Results

Expected Values

- The expected signal pattern in a cell with the typical pattern for the LSI EGR1 probe target is 2 orange and 2 green signals (2R2G). The values used to determine the upper reference limit of 6% were the number of observed 1R2G patterns per 200 scoreable nuclei. Refer to **Table 3**.

Table 3. Upper Reference Limit

Probe/Color	Atypical FISH Signal Pattern	Upper Reference Limit: Number of Patterns per 200 Nuclei
LSI EGR1/orange (R) D5S23, D5S721/green (G)	1R2G	> 12

- Individual laboratories must verify that the upper reference limit (6%) is appropriate for the laboratory's patient population.
- One orange and 2 green signals are expected in an atypical cell with loss of 1 copy of the LSI EGR1 probe target and retention of both copies of the LSI D5S23, D5S721 region. Cited published literature showed the following range of values for 5q- specimens with a 1R2G atypical signal pattern:

Publication	Disease	5q- (1R2G)	Number of specimens	Total number of specimens tested
Vance[4]	AML	45 – 91%*	8	181
Galvan[1]	MDS	35 – 81.5%	6	28
	t-MDS	25 – 75%	3	28
	t-MDS & MM	76%	1	28
	AML	20 – 99%	13	28

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; t-MDS: therapy related MDS; MM: multiple myeloma
* Based on unpublished data.

LIMITATIONS OF THE PROCEDURE

- FOR IN VITRO DIAGNOSTIC USE ONLY.
- The Vysis EGR1 FISH Probe Kit – SC is intended to be used in combination with additional biomarkers, morphology, and other clinical information.
- If a specimen has an overall low signal intensity FISH pattern and a low level of cells with an atypical (1R2G) FISH signal 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 atypical 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 SpectrumOrange/D5S23, D5S721 SpectrumGreen Probes for their respective chromosome target loci was established using metaphase chromosomes prepared from peripheral blood cultures of 5 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 1 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

		No. of Metaphase Chromosome Signals			
Probe	Correct Target Locus	Hybridized to the Correct Target Locus	Total Hybridized Signals	Analytical Specificity (%)	95% Confidence Interval
D5S23, D5S721	5p15.2	200	200	100	(98, 100)
EGR1	5q31	200	200	100	(98, 100)

Analytical Sensitivity

Analytical sensitivity is defined as the percentage of scoreable interphase nuclei with the expected typical signal pattern. The expected typical interphase signal pattern for the probes in the Vysis EGR1 FISH Probe Kit – SC 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 2 technologists. Each technologist evaluated 100 nuclei per specimen for a total of 200 nuclei per specimen and 5000 scoreable nuclei from the 25 specimens.

The analytical sensitivity was calculated as the percentage of scoreable interphase nuclei with the expected 2R2G signal pattern.

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

Table 5. Analytical Sensitivity

	No. of Interphase Chromosome Signals		Analytical Sensitivity	
Probe Kit	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 Upper Reference Limit

The upper reference limit is defined as the maximum quantity of scoreable interphase nuclei with an atypical signal pattern from either karyotypically normal specimens or 5p15.2 and 5q31 deletion-free specimens. The upper reference limit is expressed in terms of a percentage or the actual number of an atypical nuclear FISH signal pattern per the standard number of nuclei tested.

The upper reference limit for this assay is 6% or 12 1R2G patterns per 200 scoreable interphase nuclei. Specimens exceeding 12 1R2G atypical FISH signal pattern per 200 scoreable nuclei are considered as having loss of the of the Vysis LSI EGR1 probe target on chromosome 5q. 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 2 technologists evaluated 100 nuclei per specimen. Among the 25 specimens, none produced 1R2G signals at or above the 6% upper reference limit.

Reproducibility

Two replicates of the assay were run on 2 high-positive, 2 low-positive, and 2 negative panel members at 3 sites on 5 different days. The positive panel members 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 positivity. Results shown in **Table 6** show the overall agreement with the negative/positive status of the test panel members. All sites obtained 100% agreement with the known status of all 6 panel members on all 5 days, except 1 site which had 1 discordant result for a negative panel member.

Table 6. Overall Agreement, Site to Site

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

The mean and standard deviation of the percentage of cells with the 1R2G signal pattern were calculated.

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[a]	Within Day SD[b]	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
Negative 1	30	0.7	0.71	0.00	0.68	0.99
Negative 2	30	0.9	0.66	1.42	0.22	1.59

[a] Percentage of cells with 1R2G signal patterns
[b] SD = standard deviation

Using the same panel members from the site-to-site study, 4 replicates of the assay were run on 2 high-positive, 2 low-positive, and 2 negative panel members using 3 different lots of probe at a single site. The overall agreement with the known negative/positive status of the test panel members is shown in **Table 8**. All replicates using the 3 probe lots for each of the 6 panel members produced agreement with the known status of the panel members.

Table 8. Overall Agreement, Lot to Lot

	Number			
Category	**Agree**	**Disagree**	**Total**	**Percent Agreement**
High Positive	24	0	24	100
Low Positive	24	0	24	100
Negative	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**[a]	**Within Lot SD**[b]	**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
Negative 1	12	0.0	0.00	0.00	0.00
Negative 2	12	0.1	0.14	0.20	0.25

[a] Percentage of cells with 1R2G signal patterns
[b] SD = standard deviation

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

Summary of Results from Cited Published Literature

Cited published literature may discuss device uses that have not been approved or cleared by FDA.

Data from supporting literature

Literature Reference	**Population Studied**	**Number and Type of Specimens**	**Device Used**	**Observed EGR1 Results**
Sun et al[8]	Known del(5q) having MDS or AML	51 bone marrow specimens	Vysis LSI EGR1/D5S23, D5S721 probes	Overall—del(5q) was detected in 49/51 specimens • 8/8 5q-syndrome • 6/6 refractory cytopenia with multilineage dysplasia • 8/8 refractory anemia with excess blasts • 1/1 MDS, unclassifiable • 1/1 MDS, therapy-related • 5/6 MDS/MPD overlap syndrome, unclassifiable • 20/21 AML
Sun et al[8]	Known or suspected of having MDS or AML	269 bone marrow specimens	Vysis LSI EGR1/D5S23, D5S721 probes	Overall—del(5q) was detected in 17/17 specimens with cytogenetically identified del(5q). FISH identified del(5q) in 5 specimens not detected by metaphase cytogenetics - 3 cases with no analyzable metaphase and 2 cases with fewer than the required 20 metaphase.
Galvan et al[1]	MDS, therapy-related MDS (t-MDS) and AML patients	28 bone marrow specimens	Vysis LSI EGR1/D5S23, D5S721 probes	Overall 1R2G FISH pattern associated with del(5q) was detected in 23/28 specimens • 6 MDS • 3 t-MDS • 1 t-MDS and MM • 13 AML **NOTE: Atypical patterns (ie 1R3G) other than 1 orange, 2 green FISH signals were observed in 2 specimens.**
Vance et al[4]	AML	181 bone marrow specimens*	Vysis LSI EGR1/D5S23, D5S721 probes	Overall 1R2G FISH pattern associated with del(5q) was detected in 8/181 patient specimens. **NOTE: Atypical patterns other than 1 orange, 2 green FISH signals were observed in 2 specimens.**

* Based on unpublished data.

BIBLIOGRAPHY

1. Galvan AB, Mallo M, Arenillas L, et al. Does monosomy 5 really exist in myelodysplastic syndromes and acute myeloid leukemia? *Leuk Res* 2010;34:1242-1245.
2. Lai F, Godley LA, Joslin J, et al. Transcript map and comparative analysis of the 1.5-Mb commonly deleted segment of human 5q31 in malignant myeloid diseases with a del(5q). *Genomics.* 2001;71(2):235-45.
3. Joslin JM, Fernald AA, Tennant TR, et al. Haploinsufficiency of EGR1, a candidate gene in the del(5q), leads to the development of myeloid disorders. *Blood.* 2007;110(2):719-26.
4. Vance GH, Kim H, Hicks GA, et al. 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). *Leuk Res.* 2007;31:605-9.
5. Herry A, Douet-Guilbert N, Morel F, et al. Redefining monosomy 5 by molecular cytogenetics in 23 patients with MDS/AML. *Eur J Haematol.* 2007;78:457-67.
6. Bram S, Swolin B, Rodjer S, et al. Is monosomy 5 an uncommon aberration? Fluorescence in situ hybridization reveals translocations and deletions in myelodysplastic syndromes or acute myelocytic leukemia. *Cancer Genet Cytogenet.* 2003;142:107-14.
7. Bram S, Rodjer S, Swolin B. Several chromosomes involved in translocations with chromosome 5 shown with fluorescence in situ hybridization in patients with malignant myeloid disorders. *Cancer Genet Cytogenet.* 2004;155:74-8.
8. Sun Y, Cook JR. Fluorescence in situ hybridization for del(5q) in myelodysplasia/acute myeloid leukemia: Comparison of EGR1 vs. DSF1R probes and diagnostic yield over metaphase cytogenetics alone. *Leuk Res* 2010;34:340-343.
9. Genome Bioinformatics Group of UC Santa Cruz. The UCSC Genome Browser. © The Regents of the University of California. Available at: http://genome.ucsc.edu/cgi-bin/hgGateway?hgsid =185806115&clade=mammal&org=Human&db=hg18. Accessed [February 9, 2011].
10. Wiktor AE, Van Dyke DL, Stupca PJ, et al. Preclinical validation of fluorescence in situ hybridization assays for clinical practice. *Genet Med.* 2006;8:16-23.
11. US Department of Health and Human Services. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. Washington, DC: US Government Printing Office; December 2009. [Also available online. *Type* > www.cdc.gov, *search* > BMBL5 > *look up* sections III and IV.]
12. US Department of Labor, Occupational Safety and Health Administration. 29 CFR Part 1910.1030. *Bloodborne Pathogens*.
13. Clinical and Laboratory Standards Institute. *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline—Third Edition*. CLSI Document M29-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2005.
14. World Health Organization. *Laboratory Biosafety Manual*. 3rd ed. Geneva, Switzerland: World Health Organization; 2004.
15. The Association of Genetic Technologists. *The AGT Cytogenetics Laboratory Manual*. Third edition. Barch MJ, Knutsen T, Spurbeck JL, eds. Philadelphia, PA: Lippincott-Raven Publishers; 1997.

TECHNICAL ASSISTANCE

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Abbott Molecular Inc. is the legal manufacturer of the Vysis EGR1 FISH Probe Kit – SC.

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