

Vysis D7S486/CEP 7 FISH Probe Kit

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REF 04N78-020

30-608510/R1

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Key to Symbols Used	
	Manufacturer
	Reference Number
	Lot Number
	In Vitro Diagnostic Medical Device
	Global Trade Item Number
	Contains sufficient for <n> tests
	Temperature Limit
	Caution, consult accompanying documents
	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
	Consult instructions for use
	Use By
	Authorized Representative in the European Community

INTENDED USE

The Vysis D7S486/CEP 7 FISH Probe Kit is a device intended for specimen characterization and detects the LSI D7S486 probe target on chromosome 7q31 and the CEP 7 probe target on chromosome 7p11.1-q11.1 in bone marrow and peripheral blood 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 device for diagnosis, prognosis, monitoring, or risk assessment has not been established.

SUMMARY AND EXPLANATION OF THE TEST

Atypical FISH signal patterns of 1R1G and 1R2G are recurring abnormalities in several hematologic malignancies. Vance et al¹ demonstrated the Vysis LSI D7S486 SpectrumOrange/CEP 7 SpectrumGreen Probes detected a 1R2G signal pattern of the locus specific identifier (LSI) D7S486 probe target on chromosome 7q or 1R1G signal pattern on chromosome 7 (monosomy 7) in a series of bone marrow and blood specimens from untreated acute myeloid leukemia (AML) patients. In the study, 179 bone marrow specimens and 47 peripheral blood specimens were tested. Among the 179 bone marrow specimens tested, 4 bone marrow specimens were atypical for the 1R2G FISH pattern associated with the LSI D7S486 probe target on chromosome 7q with a range of 58 to 93% atypical nuclei. Among the 179 bone marrow specimens tested, 5 bone marrow specimens were atypical for the 1R1G FISH pattern with a range of 70 to 96% atypical nuclei. Among the 47 peripheral blood specimens tested, 1 peripheral blood specimen contained the 1R1G atypical FISH pattern in 11% of nuclei. An additional 3 peripheral blood specimens were atypical for the 1R2G FISH pattern with a range of 37 to 96% atypical nuclei. Cherry et al² reported the Vysis LSI D7S486 SpectrumOrange/CEP 7 SpectrumGreen Probes detected 1R2G in 3 myelodysplastic syndrome (MDS) patients with a range of 22.5 to 44% atypical bone marrow nuclei and also detected 1R1G in 2 patients with 23 and 87.5% atypical nuclei. Tefferi et al³ described 2 myelofibrosis with myeloid metaplasia (MMM) patients who had 9% and 16% bone marrow nuclei with 1R2G atypical signal patterns. A peripheral blood specimen from the MMM patient with 16% atypical bone marrow nuclei had 27% of nuclei with the 1R2G atypical signal pattern. The peripheral blood specimen from the patient with 9% 1R2G bone marrow was determined to be typical. However, the author stated that the percent of atypical nuclei may have been below detection limits in this peripheral blood specimen.

The Vysis D7S486/CEP 7 FISH probe kit uses FISH DNA probe technology to detect the probe target for LSI D7S486 (7q31) on chromosome 7q and the probe target for CEP 7 (7p11.1-q11.1) on chromosome 7.

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

DNA Probe Description

Vysis LSI D7S486 SpectrumOrange/ CEP 7 SpectrumGreen Probes

Vysis LSI D7S486 SpectrumOrange/ CEP 7 SpectrumGreen Probes are a mixture of a SpectrumOrange D7S486 probe (7q31) and a SpectrumGreen CEP 7 probe (7p11.1-q11.1). The SpectrumOrange-labeled LSI D7S486 probe is approximately 308 Kb in length (chr7: 115983468-115675366; February 2009 Assembly UCSC Human Genome Browser).⁴

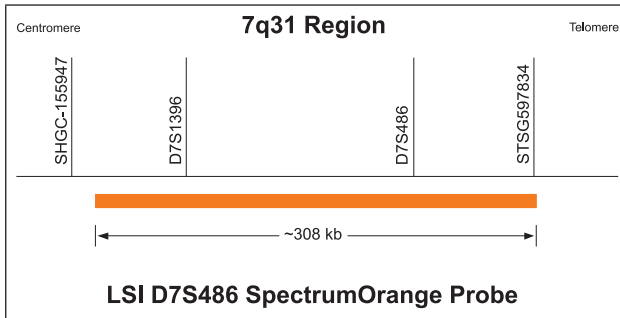
The SpectrumGreen-labeled CEP 7 probe targets the D7Z1 alpha satellite sequence at the centromere of chromosome 7.

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Vysis D7S486/CEP 7 FISH Probe Kit



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 solutions.

20X SSC Salt

20X SSC Salt is 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 utilizing appropriate controls and analytical techniques as well as taking into consideration other clinical and diagnostic test data.⁵

Bone marrow or peripheral blood cell specimens attached to microscope slides using standard cytogenetic procedures are used for this assay. The resulting specimen DNA is denatured to single-stranded form and subsequently allowed to hybridize with the probes of the Vysis D7S486/CEP 7 FISH Probe Kit. Following hybridization, the unbound probe is removed by a series of washes, and the nuclei are counterstained with DAPI II, a DNA-specific stain that fluoresces blue. Hybridization of the Vysis LSI D7S486 SpectrumOrange/CEP 7 SpectrumGreen 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 Vysis LSI D7S486 SpectrumOrange/CEP 7 SpectrumGreen probe targets, 2 orange (2R) signals (D7S486) and 2 green (2G) signals (CEP 7) will be expected.

In a cell with atypical copy numbers of the Vysis LSI D7S486 SpectrumOrange/CEP 7 SpectrumGreen probe targets, 1R1G or 1R2G will be expected.

Interphase FISH signal patterns other than 1R1G or 1R2G may be observed. Metaphase analysis may be useful in interpreting these results.

REAGENTS

Material Provided

This kit contains 5 reagents sufficient to process 20 assays. An assay is defined as one 22 mm × 22 mm Vysis LSI D7S486 SpectrumOrange/CEP 7 SpectrumGreen probe hybridization area.

Vysis LSI D7S486/CEP 7 Probes in Tris-EDTA Buffer

Part No.	30-171002
Quantity	1 vial, 20 μL/vial (100 and 200 ng/μL)
Storage	-20°C (±5°C) and protected from light
Composition	SpectrumOrange and SpectrumGreen fluorophore-labeled DNA probes in Tris-EDTA buffer

Vysis LSI/WCP Hybridization Buffer

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

DAPI II Counterstain

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

NP-40

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

20X SSC Salt

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

REAGENT STORAGE AND HANDLING INSTRUCTIONS

-15° The Vysis D7S486/CEP 7 FISH Probe Kit must be stored at -20°C (±5°C) and protected from light when not in use.

30° The NP-40 and 20X SSC Salt may be stored at -20°C (±5°C) or separately at room temperature.

- If reagents are received 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.
- This device is not for high-risk uses such as selecting therapy, predicting therapeutic response or disease screening.
- The use of this device 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 D7S486 SpectrumOrange/CEP 7 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,⁶ OSHA Standards on Bloodborne Pathogens,⁷ CLSI Document M29-A3,⁸ and other appropriate biosafety practices.⁹ 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.⁶
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.⁹

NP-40



Danger

Hazard-determining components of labeling: Triton X-100

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.

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 or attention.
P405	Store locked up.
P501	This material and its container must be disposed of in a safe way.

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

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

Handling Precautions

- The Vysis D7S486/CEP 7 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 D7S486/CEP 7 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.
- 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.
- 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 D7S486/CEP 7 FISH Probe Kit contains formamide, a teratogen. Avoid contact with skin and mucous membranes.
- Calibrated thermometers are required for measuring temperatures of solutions and water baths.
- Always verify the temperature of the wash solutions prior to each use by measuring the temperature of the solution in the Coplin jar with a calibrated thermometer.
- All hazardous materials should be disposed of according to the institution's guidelines for hazardous disposal.
- Prior to using the Vysis D7S486/CEP 7 FISH Probe Kit, verification of the upper reference limit is recommended.

ASSAY PROCEDURE

Materials Provided

- Vysis D7S486/CEP 7 FISH Probe Kit (List No. 04N78-020)

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 x 22 mm glass coverslips
- Microliter pipettor (1 to 10 µL) and sterile tips
- Timer
- Microcentrifuge
- Graduated cylinders (100 mL to 1000 mL)
- Water bath (70 to 80°C)
- Forceps
- Disposable syringe (5 mL)
- Disposable pipettes (5 mL to 20 mL)
- Coplin/Vertical Staining jars
- 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 (45 to 50°C)
- Cytogenetic drying chamber or fume hood
- ThermoBrite
- ThermoBrite humidity cards
- Microcentrifuge tubes
- Methanol/acetic acid fixative
- Phase contrast microscope
- Desiccant

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 maintenance by the manufacturer's technical representative, especially alignment of the lamp, are advised.

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.

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 D7S486 SpectrumOrange/CEP 7 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 filter(s) for use with the Vysis D7S486/CEP 7 FISH Probe Kit are the Vysis Triple Band Orange/Green/DAPI filter, the Vysis Dual Band Orange/Green Filter v2, 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 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 66g 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 pH to 5.3 ± 0.2 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.

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 7.4 ± 0.1 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

Mix thoroughly 100 mL 20X SSC Solution with 850 mL purified water in a suitable vessel. 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 pH to 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

Specimen 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 the specimen in either sodium-heparinized transport medium or a sodium-heparinized blood collection tube, such as BD Vacutainer® Blood Collection Tube.¹⁰ The manual also recommends that the specimens be transported to the cytogenetics laboratory, and cultures of the cells should be initiated immediately. Specimens should not be frozen prior to culturing or harvesting. Harvested specimens can be used immediately to prepare slides, or stored in fixative at -20°C .

NOTE: 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 20 to 30°C with a relative humidity of 30 to 50%. 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 pre-cleaned slide 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 methanol/acetic acid 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 methanol/acetic acid fixative to the remaining cell pellet before storage.
 10. Age slides using a slide warmer at 45 to 50°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 ($\pm 10^{\circ}\text{C}$) prior to hybridization or proceed to **SPECIMEN TARGET PREPARATION**.

NOTE: Fixed slides are stable at -20°C ($\pm 10^{\circ}\text{C}$) for up to 12 months. Store any remaining fixed cell pellet at -20°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 this section to ensure the materials have adequate time to thaw.

1. Transfer the specimen slides to a Coplin jar containing 70% ethanol for a minimum of 1 minute, and agitate the specimen slides within the Coplin jar for 1 to 3 seconds. Following the 70% ethanol solution, transfer to 85% ethanol for a minimum of 1 minute, and then to 100% ethanol for a minimum of 1 minute.
2. Following dehydration in ethanol, allow the slides to air dry or place the slide on a 45 to 50°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 ready to dry all slides and apply the probe mixture. If hybridizing more than 4 slides, use multiple Coplin jars to store slides in 100% ethanol until ready to dry all slides and apply the probe mixture.

Automated Probe Denaturation/Hybridization Procedure

1. Switch the power for the 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: 6 to 24 hours
 - Hyb Temperature: 37°CDenat = Denaturation
Hyb = Hybridization
3. Remove the DNA probe, Vysis LSI/WCP Hybridization Buffer, and purified water from storage and allow the reagents to reach room temperature.
4. Vortex DNA probe 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 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 the coverslip 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 the 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 ThermoBrite 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 the Coplin jar containing 0.4X SSC/0.3% NP-40 solution 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 one slide and immediately immerse the slide in the 0.4X SSC/0.3% NP-40. Agitate the specimen slide within the Coplin jar for 1 to 3 seconds. Repeat the process with the other slides up to a total of 4 slides. Start timing when the fourth slide is immersed.

NOTE: To maintain the proper temperature of 0.4X SSC/0.3% NP-40, wash 4 slides simultaneously. If there are fewer than 4 slides, add blank slides that are at room temperature to bring the total to four.

4. Remove slides after 2 minutes.
5. Immerse slides in a Coplin jar containing 2X SSC/0.1% NP-40. Agitate the specimen slides within the Coplin jar for 1 to 3 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 0.4X SSC/0.3% NP-40 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 DAPI II Counterstain 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 fluorophores
Single Band Orange	SpectrumOrange fluorophores
Dual Band Orange/Green v2	SpectrumOrange and SpectrumGreen fluorophores
Triple Band Orange/Green/DAPI	SpectrumOrange, SpectrumGreen, and DAPI 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 Target Adequacy

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

- Nuclear Morphology: Borders of cell nuclei should generally be distinguishable and 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.

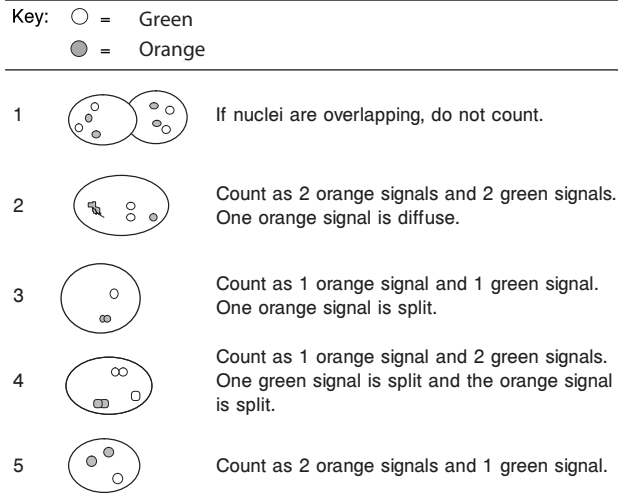
Signal Enumeration

The signal enumeration instructions are based upon a 4.5% upper reference limit for 1R1G and 6.5% upper reference limit for 1R2G, adopted from Vance et al.¹ 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 **Figure 1 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 non-specific 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 size, are counted as 1 signal. If there is a small strand of signal connecting separated signals, also count as 1 signal.
- 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.

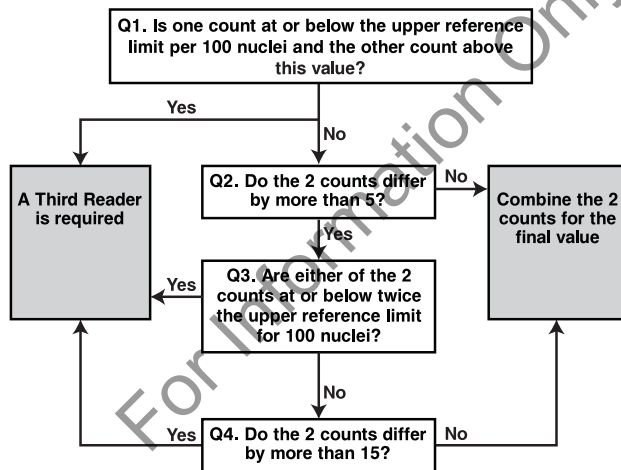
- If there is any doubt as to whether or not a cell should be scored, do not score the cell.
- If a specimen has an overall low signal intensity FISH pattern and a low level of cells with an atypical (1R2G and/or 1R1G) FISH signal pattern, use of the appropriate single pass filter to confirm the pattern is recommended. Failure to follow this recommendation may result in inaccurate identification of signals.

Figure 1. Dual Color Signal Counting Guide



After recording the results, the number of 1R1G and/or 1R2G atypical signal patterns from each reader are examined, and the following stepwise rules are applied to determine the final 200 nuclei count. In the event that both atypical signal patterns (1R1G and 1R2G) are observed, the rules are applied separately for each atypical signal pattern. The upper reference limits are adopted from Vance et al.¹ Refer to **Figure 2** for **D7S486/CEP 7 Signal Enumeration Flowchart** and **Table 1 Upper Reference Limit for Inclusion of Third Reader for the Atypical Signal Pattern**. For scenarios detailing the application of the rules in **Figure 2**, see the text following the table.

Figure 2. D7S486/CEP 7 Signal Enumeration Flowchart



Application of the Rules in Figure 2

Scenario 1:

When 1 reader has an atypical signal pattern count below the upper reference limit for 100 nuclei, and the other reader has a count above this value, 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.

Scenario 2:

When the atypical signal pattern 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.

Scenario 3:

When the atypical signal pattern 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 atypical signal pattern counts to the upper reference limit per 100 nuclei.

If either of the 2 readers have atypical signal pattern counts less than or equal to twice the upper reference limit per 100 nuclei, and if the 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.

Scenario 4:

When both of the 2 readers have atypical count values greater than twice the upper reference limit per 100 nuclei and if those counts differ by 15 or fewer per 100 nuclei, the scores will be combined to generate the count for 200 nuclei.

Scenario 5:

If the 2 readers have atypical signal pattern 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.

Scenario 6:

If a third reader is required, and if the specimen contains both the 1R1G and 1R2G patterns, the counting rules are applied to each pattern independently. This means that the counts from the second and third reader might be combined for a total of 200 cells for the 1R1G pattern while the counts for the first and second reader might be combined for a total of 200 cells for the 1R2G pattern.

For example, the first and second reader scores are:

Signal Patterns	2R2G	1R1G	1R2G	Total
Reader 1	77	8	15	100
Reader 2	86	1	13	100

Because the count for Reader 1 for the 1R1G is above and the count for Reader 2 is below the 100 nuclei upper reference limit, a third reader is required. The third reader scores are:

Signal Patterns	2R2G	1R1G	1R2G	Total
Reader 3	89	4	7	100

For the 1R1G pattern, Reader 2 and Reader 3 scores are the closest. Therefore, these scores are combined for a total of five 1R1G atypical counts in 200 nuclei:

Signal Patterns	2R2G	1R1G	1R2G	Total
Reader 2	86	1	13	100
Reader 3	89	4	7	100
Total	N/A	5	N/A	200

For the 1R2G pattern, the third reader was not required. Therefore, the scores are combined for a total of 28 1R2G atypical counts in 200 nuclei:

Signal Patterns	2R2G	1R1G	1R2G	Total
Reader 1	77	8	15	100
Reader 2	86	1	13	100
Total	N/A	N/A	28	200

Table 1. Upper Reference Limits for Inclusion of Third Reader for the Atypical Signal Pattern

Probe/Color	Atypical FISH Pattern	Upper Reference Limit per 100 Nuclei
D7S486(Orange)/CEP 7 (Green)	1R1G	> 4.5 ^a
D7S486(Orange)/CEP 7 (Green)	1R2G	> 6.5 ^a

^a The fractional upper reference limit is half the value of the upper reference limit per 200 nuclei.

INTERPRETATION OF RESULTS

Expected Values

The expected signal pattern in a cell with the typical pattern for the LSI D7S486/CEP 7 probe target is 2 orange and 2 green signals (2R2G). The values used for the upper reference limits of 4.5% for 1R1G and 6.5% for 1R2G were the number of observed 1R1G and 1R2G patterns per 200 scoreable nuclei.¹ Refer to **Table 2 Upper Reference Limits**.

Table 2. Upper Reference Limits

Probe/Color	Atypical FISH Signal Pattern	Upper Reference Limit: Number of Patterns per 200 Nuclei
D7S486(Orange)/CEP 7 (Green)	1R1G	> 9
D7S486(Orange)/CEP 7 (Green)	1R2G	> 13

- Individual laboratories must verify that the upper reference limit (4.5% and 6.5%) is appropriate for the laboratory's patient population.
- One orange and 2 green signals or 1 orange and 1 green signal are expected in an atypical cell. Cited published literature showed the following range of values for specimens with an 1R2G and 1R1G atypical signal pattern:

Publication	Disease	Specimen	1R1G	Number of 1R1G Specimens	1R2G	Number of 1R2G Specimens	Total number of specimens tested
Vance ¹	AML ^a	BM ^b	70 to 96% ^c	5	58 to 93% ^c	4	179
		PB ^b	11% ^c	1	37 to 96% ^c	3	47
Cherry ²	MDS ^a	BM ^b	23 and 87.5%	2	22.5 to 44%	3	48
Tefferi ³	MMM ^a	BM ^b	N/A ^d	N/A ^d	9 and 16%	2	42
		PB ^b	N/A ^d	N/A ^d	27%	1	

^a AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; MMM: myelofibrosis with myeloid metaplasia

^b BM: bone marrow; PB: peripheral blood

^c Based on unpublished data

^d Not applicable

LIMITATIONS OF THE PROCEDURE

- FOR IN VITRO DIAGNOSTIC USE
- The Vysis D7S486/CEP 7 FISH Probe Kit is intended to be used in combination with additional biomarkers, morphology, and other clinical information.
- Other atypical signal patterns may occur, and metaphase analysis may be helpful in characterization of such patterns.
- If a specimen has an overall low signal intensity FISH pattern and a low level of cells with an atypical (1R2G and/or 1R1G) 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.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Specificity

Analytical specificity is defined as the percentage of signals that hybridize to the correct locus and no other location.^{5,11}

The analytical specificity of the Vysis LSI D7S486 SpectrumOrange/CEP 7 SpectrumGreen Probes for their respective chromosome target loci was established using metaphase chromosomes prepared from peripheral blood cultures of 4 male and 1 female karyotypically normal specimens 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 per probe.

For each probe, 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.

For each probe, the specificity 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.^{5,11}

The analytical specificity of the Vysis D7S486/CEP 7 FISH Probe Kit was 100%, as shown in **Table 3**.

Table 3. Analytical Specificity

Probe	Correct Target Locus	No. of Metaphase Chromosome Signals		Specificity (%)	95% Confidence Interval
		Hybridized to the Correct Target Locus	Total Hybridized		
LSI D7S486	7q31	200	200	100	(98,100)
CEP 7	7p11.1-q11.1	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 D7S486/CEP 7 FISH probe kit is 2 orange (2R) and 2 green (2G) signals per nucleus.

The analytical sensitivity of the Vysis LSI D7S486 SpectrumOrange/CEP 7 SpectrumGreen Probes was established using interphase nuclei prepared from 25 bone marrow and 25 peripheral blood specimens that were either karyotypically normal or patients lacking 1R1G and 1R2G. The orange and green signal patterns of nuclei for each of the 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 for each of the specimen types.

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

The Vysis D7S486/CEP 7 FISH Probe Kit has an analytical sensitivity of 98.1% for bone marrow specimens and 98.5% for peripheral blood specimens.

Table 4. Analytical Sensitivity

Probe	Specimen ^a	No. of Interphase Chromosome Signals		Analytical Sensitivity	
		With Expected Scoreable Signal Pattern	Nuclei	Point Estimate	95% CI ^b
D7S486	BM ^b	4903	5000	98.1	(97.6,98.4)
D7S486	PB ^b	4923	5000	98.5	(98.1,98.8)

^a 25 Karyotypically normal specimens for both specimen types.

^b BM: bone marrow; PB: peripheral blood; CI: Confidence Interval

Verification of Upper Reference Limits

The upper reference limit is defined as the maximum quantity of scoreable interphase nuclei with a specific atypical signal pattern at which a specimen is considered karyotypically normal for that signal pattern. The upper reference limit is expressed in terms of a percentage or the actual number of a specific atypical nuclear FISH signal pattern per the standard number of nuclei tested.

The upper reference limit for 1R1G is 4.5% or 9 1R1G patterns per 200 scoreable interphase nuclei, and the upper reference limit for 1R2G is 6.5% or 13 1R2G patterns per 200 scoreable interphase nuclei. Specimens exceeding 9 1R1G patterns and/or 13 1R2G patterns per 200 scoreable nuclei are considered atypical for 1R1G and/or 1R2G with the Vysis D7S486/CEP 7 FISH Probe target.

The Vysis D7S486/CEP 7 FISH assay was performed on interphase nuclei from 25 bone marrow and 25 peripheral blood specimens from either karyotypically normal individuals or patients lacking 1R1G and 1R2G. The signal patterns of 200 nuclei for each specimen type were evaluated by each of 2 technologists scoring 100 nuclei per specimen.

Among the 25 karyotypically normal specimens for both peripheral blood and bone marrow, none produced 1R1G and 1R2G signals above the 4.5% and 6.5% upper reference limits.

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 non-consecutive days for each specimen type. The positive panel members for the site-to-site study were obtained by mixing positive cells with normal cells, for each of the specimen types, to obtain the desired levels of positivity. Results shown in **Tables 5 through 8** show the overall agreement with the negative/positive status of the test panel members.

All 3 sites demonstrated 100% agreement with the known status of the negative and high positive panel members for both specimen types and both signal patterns. For the bone marrow specimens, the low positives demonstrated 88% (1R2G) and 97% (1R1G) agreement. For the peripheral blood, the low positives demonstrated 95% (1R2G) and 93% (1R1G) agreement.

Table 5. Overall Agreement, Site-to-Site—For Specimen type BM and signal 1R2G

Category	Number			Percent Agreement (%)
	Agree ^a	Disagree ^b	Total	
Negative	60	0	60	100%
Low Positive	53	7	60	88%
High Positive	60	0	60	100%

^a Agree is number of concordant slides

^b Disagree is number of discordant slides

Table 6. Overall Agreement, Site-to-Site—For Specimen type BM and signal 1R1G

Category	Number			Percent Agreement (%)
	Agree ^a	Disagree ^b	Total	
Negative	60	0	60	100%
Low Positive	58	2	60	97%
High Positive	60	0	60	100%

^a Agree is number of concordant slides

^b Disagree is number of discordant slides

Table 7. Overall Agreement, Site-to-Site—For Specimen type PB and signal 1R2G

Category	Number			Percent Agreement (%)
	Agree ^a	Disagree ^b	Total	
Negative	60	0	60	100%
Low Positive	57	3	60	95%
High Positive	60	0	60	100%

^a Agree is number of concordant slides

^b Disagree is number of discordant slides

Table 8. Overall Agreement, Site-to-Site—For Specimen type PB and signal 1R1G

Category	Number			Percent Agreement (%)
	Agree ^a	Disagree ^b	Total	
Negative	60	0	60	100%
Low Positive	56	4	60	93%
High Positive	60	0	60	100%

^a Agree is number of concordant slides

^b Disagree is number of discordant slides

The mean and standard deviation (SD) of the percentage of cells with the 1R2G and 1R1G signal patterns were calculated.

The analysis of variance components for the site-to-site study is shown in Tables 9 through 12.

Table 9. Site-to-Site Analysis of Variance Components—For Specimen type BM and signal 1R2G

Sample	N	Mean	Within-Day	Between-Day	Between-Site	Total
			(Comp.)	(Comp.)	(Comp.)	
Negative 1	30	0.5	0.27	0.32	0.06	0.43
Negative 2	30	0.4	0.38	0.14	0.24	0.47
Low Positive 1	30	10.5	2.11	1.41	1.14	2.78
Low Positive 2	30	10.0	3.19	0.00	2.33	3.95
High Positive 1	30	43.9	6.76	0.00	7.62	10.19
High Positive 2	30	42.0	4.61	0.00	5.44	7.13

Table 10. Site-to-Site Analysis of Variance Components—For Specimen type BM and signal 1R1G

Sample	N	Mean	Within-Day	Between-Day	Between-Site	Total
			(Comp.)	(Comp.)	(Comp.)	
Negative 1	30	0.5	0.67	0.14	0.49	0.84
Negative 2	30	0.3	0.37	0.00	0.25	0.44
Low Positive 1	30	8.9	2.61	0.00	1.19	2.87
Low Positive 2	30	9.3	2.43	0.00	0.00	2.43
High Positive 1	30	48.3	6.20	1.30	8.06	10.25
High Positive 2	30	43.9	3.78	3.97	4.19	6.90

Table 11. Site-to-Site Analysis of Variance Components—For Specimen type PB and signal 1R2G

Sample	N	Mean	Within-Day	Between-Day	Between-Site	Total
			(Comp.)	(Comp.)	(Comp.)	
Negative 1	30	0.4	0.53	0.27	0.38	0.71
Negative 2	30	0.4	0.42	0.25	0.00	0.49
Low Positive 1	30	10.4	2.46	0.00	2.03	3.19
Low Positive 2	30	12.5	2.57	0.00	0.38	2.60
High Positive 1	30	42.1	3.26	2.05	5.82	6.98
High Positive 2	30	52.8	4.07	1.82	2.15	4.95

Table 12. Site-to-Site Analysis of Variance Components—For Specimen type PB and signal 1R1G

Sample	N	Mean	Within-Day	Between-Day	Between-Site	Total
			(Comp.)	(Comp.)	(Comp.)	
Negative 1	30	0.3	0.65	0.00	0.32	0.72
Negative 2	30	0.4	0.47	0.20	0.25	0.57
Low Positive 1	30	9.1	2.61	0.00	0.55	2.67
Low Positive 2	30	6.9	2.35	0.52	0.27	2.42
High Positive 1	30	44.8	3.77	0.00	5.35	6.55
High Positive 2	30	38.8	3.90	3.17	1.63	5.29

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 for each specimen type. The overall agreement with the known negative/positive status of the test panel members are shown in Tables 13 through 16.

All 3 lots demonstrated 100% agreement with the known status of the negative and high positive panel members for both specimen types and both signal patterns. For the bone marrow specimens, the low positives demonstrated 88% (1R2G) and 92% (1R1G) agreement. For the peripheral blood, the low positives demonstrated 100% (1R2G) and 96% (1R1G) agreement.

Table 13. Overall Agreement, Lot-to-Lot—For Specimen type BM and signal 1R2G

Category	Number			Percent Agreement (%)
	Agree ^a	Disagree ^b	Total	
Negative	24	0	24	100%
Low Positive	21	3	24	88%
High Positive	24	0	24	100%

^a Agree is number of concordant slides

^b Disagree is number of discordant slides

Table 14. Overall Agreement, Lot-to-Lot—For Specimen type BM and signal 1R1G

Category	Number			Percent Agreement (%)
	Agree ^a	Disagree ^b	Total	
Negative	24	0	24	100%
Low Positive	22	2	24	92%
High Positive	24	0	24	100%

^a Agree is number of concordant slides

^b Disagree is number of discordant slides

Table 15. Overall Agreement, Lot-to-Lot—For Specimen type PB and signal 1R2G

Category	Number			Percent Agreement (%)
	Agree ^a	Disagree ^b	Total	
Negative	24	0	24	100%
Low Positive	24	0	24	100%
High Positive	24	0	24	100%

^a Agree is number of concordant slides

^b Disagree is number of discordant slides

Table 16. Overall Agreement, Lot-to-Lot—For Specimen type PB and signal 1R1G

Category	Number			Percent Agreement (%)
	Agree ^a	Disagree ^b	Total	
Negative	24	0	24	100%
Low Positive	23	1	24	96%
High Positive	24	0	24	100%

^a Agree is number of concordant slides

^b Disagree is number of discordant slides

The analysis of variance components for the lot-to-lot study is shown in **Tables 17 through 20.**

Table 17. Lot-to-Lot Analysis of Variance Components—For Specimen type BM and signal 1R2G

Sample	N	Mean	Within-Lot	Between-Lot	Total
			(Comp.)	(Comp.)	
			SD	SD	SD
Negative 1	12	0.1	0.22	0.06	0.23
Negative 2	12	0.2	0.28	0.00	0.28
Low Positive 1	12	8.7	2.31	1.63	2.82
Low Positive 2	12	11.3	1.58	0.00	1.58
High Positive 1	12	47.8	5.65	2.49	6.17
High Positive 2	12	48.9	3.53	2.66	4.42

Table 18. Lot-to-Lot Analysis of Variance Components—For Specimen type BM and signal 1R1G

Sample	N	Mean	Within-Lot	Between-Lot	Total
			(Comp.)	(Comp.)	
			SD	SD	SD
Negative 1	12	0.1	0.22	0.06	0.23
Negative 2	12	0.0	0.14	0.00	0.14
Low Positive 1	12	6.8	2.58	0.88	2.73
Low Positive 2	12	8.1	1.16	0.00	1.16
High Positive 1	12	43.9	6.55	4.86	8.16
High Positive 2	12	38.5	4.03	3.71	5.48

Table 19. Lot-to-Lot Analysis of Variance Components—For Specimen type PB and signal 1R2G

Sample	N	Mean	Within-Lot	Between-Lot	Total
			(Comp.)	(Comp.)	
			SD	SD	SD
Negative 1	12	0.1	0.22	0.06	0.23
Negative 2	12	0.1	0.20	0.00	0.20
Low Positive 1	12	11.6	2.14	0.98	2.35
Low Positive 2	12	11.0	1.99	0.00	1.99
High Positive 1	12	46.5	2.47	1.52	2.89
High Positive 2	12	58.1	3.38	0.00	3.38

Table 20. Lot-to-Lot Analysis of Variance Components—For Specimen type PB and signal 1R1G

Sample	N	Mean	Within-Lot	Between-Lot	Total
			(Comp.)	(Comp.)	
			SD	SD	SD
Negative 1	12	0.0	0.00	0.00	0.00
Negative 2	12	0.0	0.14	0.00	0.14
Low Positive 1	12	9.1	2.05	1.76	2.70
Low Positive 2	12	5.7	1.22	0.00	1.22
High Positive 1	12	37.1	2.87	0.90	3.01
High Positive 2	12	32.0	3.42	1.69	3.81

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 D7S486/CEP 7 Results
Vance et al ¹	AML ^a	179 bone marrow and 47 peripheral blood specimens ^b	Vysis LSI D7S486/CEP 7 probes	Overall 1R2G signal pattern was detected in 4/179 bone marrow specimens and 3/47 peripheral blood specimens. Overall 1R1G signal pattern was detected in 5/179 bone marrow specimens and 1/47 peripheral blood specimens.
Cherry et al ²	MDS ^a	48 bone marrow specimens	Vysis LSI D7S486/CEP 7 probes	Overall 1R2G signal pattern was detected in 3/48 bone marrow specimens. Overall 1R1G signal pattern was detected in 2/48 bone marrow specimens.
Tefferi et al ³	MMM ^a	42 bone marrow and peripheral blood specimens	Vysis LSI D7S486/CEP 7 probes	Overall 1R2G signal pattern was detected in 2/42 bone marrow specimens and 1/42 peripheral blood specimens.

^a AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; MMM: myelofibrosis with myeloid metaplasia

^b Based on unpublished data.

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TECHNICAL ASSISTANCE


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