CEP 8 SpectrumOrange DNA Probe Kit

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REF 07J20-008
07J22-008

30-608315/R3

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DNA Probe Kit
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Key to Symbols Used

	Manufacturer
REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
Σ	Contains sufficient for <n> tests
	Temperature Limitation
	Danger
	Danger
	Danger
	Biological Risks
	Consult instructions for use
	Used By
EC REP	Authorized Representative in the European Community

CEP 8 SPECTRUMORANGE DIRECT LABELED CHROMOSOME ENUMERATION DNA PROBE KIT FOR FLOURESCENCE IN SITU HYBRIDIZATION

(Part No. 30-160008, List No. 07J20-008;
Part No. 32-160008, List No. 07J22-008)

PROPRIETARY NAME

CEP 8 SpectrumOrange Direct Labeled Fluorescent DNA Probe Kit

COMMON OR USUAL NAME

Fluorescence in situ hybridization (FISH) reagents

INTENDED USE

The CEP 8 SpectrumOrange DNA Probe Kit is intended to detect AT rich alpha satellite sequences in the centromere region of chromosome 8 in conjunction with routine diagnostic cytogenetic testing. It is indicated for use as an adjunct to standard cytogenetic analysis for identifying and enumerating chromosome 8 via fluorescence in situ hybridization (FISH) in interphase nuclei and in metaphase spreads of cells obtained from bone marrow in patients with myeloid disorders [Chronic myelogenous leukemia (CML), Acute myeloid leukemia (AML), Myeloproliferative disorder (MPD), Myelodysplastic syndrome (MDS), and Hematological disorders not otherwise specified (HDNOS)]. It is not intended to be used as a stand-alone assay for test reporting. It is not intended for use in long term cell cultured materials such as amniocytes, fibroblasts, and tumor cells.

SUMMARY AND EXPLANATION

Chromosome aberrations in myeloid disorders have been extensively studied; many different chromosomal abnormalities occur in these disorders. Approximately 18% of all myeloid disorders involve trisomy 8.[1] CML accounts for 15% of adult leukemias. The median age of disease onset is 67 years; however CML occurs in all age groups (SEER statistics). In 2012, an estimated 4,870 cases will be diagnosed in the USA, and 440 patients will die from the disease.[16] CML is a clonal bone marrow disease characterized by neoplastic overproduction of granulocytes. The disease has 2 major phases: the initial chronic phase and the terminal blast crisis phase. The transition from chronic to blast phase is often accompanied by 1 or more of 3 specific chromosomal abnormalities: trisomy 8, an additional Philadelphia chromosome, or an isochromosome of 17q. These abnormalities often precede the clinical blast phase by 2 to 6 months. Trisomy 8 is present in approximately 20 to 50% of the abnormal cases.[2-4]

MPD represent a group of chronic, clonal, neoplastic disorders characterized by the excessive proliferation of 1 or more lineages of the myeloid series. Maturation is generally less severely affected; no maturational arrest is present. The principal subtypes of MPD are polycythemia vera (PV), idiopathic myelofibrosis (IMF), essential thrombocythemia (ET), and CML, which is usually recognized as a separate entity and is described above. The various MPD subtypes differ with respect to the hematopoietic cell type predominantly affected. All of the myeloproliferative disorders have a tendency to progress to acute nonlymphocytic leukemia (ANLL). Although no karyotypic abnormality is specific for MPD, certain chromosomes are involved preferentially in numerical and structural abnormalities. The most common changes are rearrangements of the long arm of chromosome 1, monosomy 7, trisomy 8, trisomy 9, a deletion on 13q, and a deletion on 20q. Trisomy 8 accounts for approximately 20% of all chromosomal abnormalities in PV cases and usually occurs with trisomy 9.[5,6] Among chromosomally abnormal IMF cases, trisomy 8 is present in 10%.[4] The MDS are a group of poorly defined disorders, mostly affecting the elderly, in which bone marrow dysfunction is caused by both qualitative and quantitative defects of hematopoietic cells. The MDS represent myeloid clonal hemopathies with relatively heterogeneous spectrums of presentation. The major clinical problems in these disorders are morbidities caused by patients' cytopenias and the potential for MDS to evolve into AML. In the general population, MDS occur in 5 per 100,000 people. However, among individuals older than age 70, the incidence increases between 22 and 45 per 100,000 and increases further with age.[17] The most important morphological bone marrow feature in MDS is a disturbance in the differentiation and proliferation of all cell lineages. The patients exhibit peripheral cytopenia despite bone marrow normocellularity or hypercellularity. Nonrandom chromosomal aberrations have been observed in up to 79% of consecutively karyotyped MDS patients. The most frequent aberrations are a deletion on 5q, monosomy 7, and trisomy 8.[4] Trisomy 8 occurs in approximately 10 to 20% of the chromosomally abnormal cases.[4,7,8]

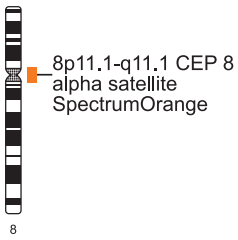
ANLL is the most common form of leukemia in adults, affecting approximately 2.5 persons per 100,000 per year. The salient pathological feature of ANLL is the excessive accumulation of immature nonlymphocytic bone marrow precursor cells in the marrow itself, in peripheral blood, and sometimes in other tissues. A postulated block in precursor cell maturation prevents further passage of myeloid cells down 1 or more of the 4 main differentiation pathways. More than 25 cytogenetic abnormalities have been associated with ANLL, with 80% of ANLL patients having at least 1 of them. This finding suggests that the disease results from the unrestrained expansion of a single

clone originating from 1 genetically rearranged cell. Trisomy 8 is by far the most frequent numerical aberration in ANLL, occurring as a solitary change in 7% of all chromosomally abnormal cases and in 15% of abnormal cases overall.[2,4,9]

PRINCIPLES OF THE PROCEDURE

In situ hybridization is a technique that allows the visualization of specific nucleic acid sequences within a cellular preparation. Specifically, DNA FISH involves the precise annealing of a single-stranded, fluorescently-labeled DNA probe to complementary target sequences. The hybridization of the probe with the cellular DNA site is visible by direct detection using fluorescence microscopy.

Tissue consisting of interphase nuclei or metaphase spreads are attached to glass slides using standard cytogenetic protocols. The resulting specimen DNA is denatured to its single-stranded form and then allowed to hybridize with the CEP 8 DNA probe. Following hybridization, the excess and unbound probe is removed by a series of washes and the chromosomes and nuclei are counterstained with the DNA-specific stain DAPI (4,6 diamidino-2-phenylindole) that fluoresces blue. Hybridization of the CEP 8 DNA probe is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters allowing visualization of both the intense orange fluorescent signal concentrated at the centromere of chromosome 8 and the blue counterstained chromosome and nuclei. The enumeration of chromosome 8 is conducted by microscopic examination of interphase nuclei and/or metaphase spreads. The fluorescently stained centromeres of chromosome 8 stand out brightly against the general blue fluorescence of the nuclear DNA provided by the DAPI counterstain. Relative to standard cytogenetic methods, the CEP procedure provides a higher percentage of interpretable nuclei per slide and enables visual enumeration of chromosome 8 within the nuclei. The assay results are reported as the percentage of nuclei with 0, 1, 2, 3, 4, and >4 fluorescent signals. Each fluorescent signal corresponds to the centromere of a chromosome 8. A nucleus exhibiting 2 fluorescent signals is referred to as “bi-signaled”. A nucleus exhibiting 3 fluorescent signals is referred to as “tri-signaled”.



The CEP 8 DNA probe is a SpectrumOrange directly-labeled fluorescent DNA probe specific for the AT rich alpha satellite DNA sequence at the centromeric region of chromosome 8 (8p11.1-q11.1). This assay is designed for the detection and quantification of chromosome 8 in both interphase nuclei and metaphase spreads by FISH.

REAGENTS AND INSTRUMENTS

Materials Provided

This kit contains 4 reagents sufficient to process approximately 20 assays. An assay is defined as one 22 mm × 22 mm target area.

Table 1. CEP 8 SpectrumOrange DNA Probe Kit (Part No. 30-160008, List No. 07J20-008)

Component	Composition	Part No.	Contents	Storage
CEP 8 DNA Probe: E. coli plasmid (The probe is predenatured)	5 ng/µL SpectrumOrange fluorophore-labeled alpha satellite DNA probe premixed with Hybridization Buffer. (dextran sulfate, formamide SSC)	30-170008	220 µL/ 1 vial	–20°C protect from light.
DAPI II Counterstain	125 ng/mL DAPI (4,6-diamidino-2-phenylindole) in phenylenediamine dihydrochloride, glycerol, and buffer.	30-804841	300 µL/ 1 vial	–20°C protect from light.
NP-40	nonionic detergent	30-804818	1 mL/1 vial	–25 to 30°C
20X SSC	sodium chloride and sodium citrate	30-805850	66 g/1 container	–20 to 25°C

Table 2. CEP 8 SpectrumOrange DNA Probe Kit with ProbeChek Control Slides (Part No. 32-160008, List No. 07J22-008)

Kit contents include the following ProbeChek slides in addition to the components cited in **Table 1**.

Component	Composition	Part No.	Contents	Storage
ProbeChek Negative Control Slides 0% Trisomy 8/12	Fixed biological specimen derived from normal (~0% trisomy 8/12) cultured human lymphoblast cells on microscope slides	30-805000	5 slides (10 target areas)	–20°C, desiccated
ProbeChek Positive Control Slides 10% Trisomy 8/12	Fixed biological specimen derived from a mixture of (~10% trisomy 8/12 and ~90% disomy 8/12) cultured human lymphoblast cells on microscope slides	30-805002	5 slides (10 target areas)	–20°C, desiccated

STORAGE AND HANDLING

Store the CEP 8 DNA probe kit as a unit at –20°C protected from light and humidity. The ProbeChek Control Slides should be stored –20°C in a sealed container with desiccant to protect them from humidity. After preparation of the solutions from the 20X SSC salts and the NP-40 components, the solutions may be stored at room temperature for up to 6 months. Expiration dates for each of the kit components are indicated on each individual kit component.

Materials Required But Not Provided

Laboratory Reagents

NOTE: Where storage conditions are not listed in this insert or the product label, store reagent per vendor recommendations.

- Ultra-pure grade formamide.
- Ethanol (100%). Store at room temperature.
- Concentrated (12N) HCl
- 1N NaOH
- Purified water (distilled or deionized or Milli-Q) Store at room temperature.
- Fixative (3:1 methanol:acetic acid). Prepare fresh daily.
- Drierite and Nitrogen gas
- Rubber cement

Laboratory Equipment

- Fluorescence microscope equipped with recommended filters
- Phase contrast light microscope
- Precleaned microscope slides
- Slide warmer (45 to 50°C)
- 22 mm × 22 mm glass coverslips
- Microliter pipettor (1 to 10 µL) and sterile tips
- Polypropylene microcentrifuge tubes (0.5 mL or 1.5 mL)
- Timer
- Magnetic stirrer
- Vortex mixer
- Microcentrifuge
- Graduated cylinder
- Water baths (67 ± 2°C and 73 ± 1°C)
- Air incubator (42°C)
- Diamond-tipped scribe
- Humidified chamber
- Forceps
- Disposable syringe (5 mL)
- Coplin jars (6) Suggested type: Wheaton Product. No. 900620 vertical staining jar
- pH meter and pH paper
- Calibrated thermometer
- Wire test tube racks
- 0.45 µm pore filtration unit

Microscope Equipment and Filters

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 63X or 100X oil immersion achromat type objective.

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

Filters: The Abbott Molecular line of multi-bandpass fluorescence microscope filter sets has been optimized for analysis using the CEP direct chromosome enumeration system. Complete, assembled, and aligned, these Abbott Molecular filter sets are available for most microscope models. The recommended filter for the CEP 8 SpectrumOrange DNA Probe kit is a dual-bandpass DAPI/Orange Filter that allows the simultaneous excitation and emission of the SpectrumOrange and DAPI fluorophores (blue). Contact Abbott Molecular Technical Services Department (1-800-553-7042 in the US and from outside the US +49-6122-580) for more information on Abbott Molecular filter sets.

Preparation of Working Reagent Solutions

20X SSC

To prepare, add together:

66 g	20X SSC
200 mL	Purified water
250 mL	Final Volume

Mix thoroughly. Measure pH at room temperature with a pH meter. Adjust to pH 5.3 with concentrated HCl, if necessary. Bring the total volume to 250 mL. Filter through a 0.45 µm filtration unit. Store in a covered container at room temperature for up to 6 months.

Denaturing Solution

To prepare, add together:

49 mL	Formamide
7 mL	20X SSC, pH 5.3
14 mL	Purified water
70 mL	Final Volume

Mix well and place in a glass Coplin jar. Measure pH at room temperature with a pH meter. Verify pH is between 7.0 to 8.0. Store in a covered container at 2 to 8°C. This solution can be used for up to 1 week. Check pH prior to each use.

Ethanol Wash Solutions

Prepare v/v dilutions of 70%, 85%, and 100% using 100% ethanol and purified water. Store at room temperature in tightly capped containers. Dilutions may be used for 1 week unless evaporation occurs or the solution becomes diluted due to excessive use.

0.4X SSC Wash Solution

To prepare, add together:

950 mL	Purified water
20 mL	20X SSC, pH 5.3
1000 mL	Final Volume

Mix thoroughly. Measure pH at room temperature with a pH meter. Adjust to pH 7.0 to 7.5, if necessary. Adjust volume to 1 liter with water. Filter through 0.45 µm pore filtration unit. Store unused solution in a covered container at room temperature for up to 6 months. Discard solution that was used in the assay at the end of each day.

0.1% NP-40 in 2X SSC Wash Solution

To prepare, add together:

100 mL	20X SSC, pH 5.3
849 mL	Purified water
1 mL	NP-40
1000 mL	Final Volume

Mix thoroughly. Measure pH at room temperature with a pH meter. Adjust the pH to 7.0 to 7.5 with 1N NaOH. Adjust volume to 1 liter with water. Filter through 0.45 µm pore filtration unit. Add 70 mL to a Coplin jar and maintain at room temperature. Store unused solution in a covered container at room temperature for up to 6 months. Discard solution that was used in the assay at the end of each day.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

1. For In Vitro Diagnostic Use
2. All biological specimens should be treated as if capable of transmitting infectious agents. The control slides provided with this kit are manufactured from human-cultured lymphoblast cells that have been fixed in a solution of methanol:acetic acid (3:1, v:v). Because it is often impossible to know which might be infectious, all human specimens and control slides should be treated with universal precautions. Guidelines for specimen handling are available from the US Centers for Disease Control and Prevention.[11]
3. Hybridization conditions may be adversely affected by the use of reagents other than those provided or recommended by Abbott Molecular.
4. Failure to follow all procedures for slide denaturation and hybridization may cause unacceptable or erroneous results.
5. Fluorophores are readily photobleached by exposure to light. To limit this degradation, handle all solutions 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.
6. CEP 8 DNA probe contains formamide, a teratogen. Avoid contact with skin and mucous membranes. Refer to MSDS for more information.
7. Calibrated thermometers are required for measuring temperatures of solutions, water baths, and incubators.
8. All hazardous materials should be disposed of according to your institution's guidelines for hazardous disposal.

CEP 8 SpectrumOrange DNA Probe

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,[18] OSHA Standards on Bloodborne Pathogens,[19] CLSI Document M29-A3,[20] and other appropriate biosafety practices.[21] 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.[18]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[21]

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.
H318	Causes serious eye damage.
H412	Harmful 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.
P501	This material and its container must be disposed of in a safe way.

Material Safety Data Sheets (MSDS) for all reagents provided in the kits are available upon request from the Abbott Molecular Technical Services Department (1-800-553-7042 in the US and from outside of the US +49-6122-580).

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 ACT Cytogenetics Laboratory Manual contains recommendations for specimen collection and culturing. The ACT recommends collection of bone marrow in either sodium-heparinized transport medium or a sodium-heparinized vacutainer. According to the ACT Manual, both containers are acceptable.[2]

The ACT Manual recommends that the specimens be transported to the cytogenetics laboratory and direct preparations or cultures of the bone marrow should be initiated immediately. The ACT Manual contains a number of recommendations for specimen culturing, direct preparation and specimen harvesting. Harvested bone marrow can be immediately used to prepare slides, or stored in fixative at −20°C.[2]

Icteric or 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

The following method may be used for preparing slides from cultured specimens.

1. Place a water bath and humidifier within a humidity containment device that is equipped with a front access. Cover the front of the containment device loosely with plastic wrap, but do not completely block access to the interior. If the room hygrometer reading is below 45%, a humidifier should be used.
2. Prewarm the water bath to 67±2°C. Place test tube racks in the center of the water bath so that they do not touch the sides of the bath. Maintain the water level to the top of the test tube rack throughout the procedure.
3. Prepare the cell pellet with fixative so that the suspension is slightly cloudy.
4. Clean a microscope slide by flooding both sides of the slide with 70% ethanol (use a squeeze bottle). Wipe the slide dry by drawing a laboratory wipe, down the length of the slide from the labeled end. Use a pencil to label a slide with a painted blaze.
5. Dip the cleaned slide in a Coplin jar containing fixative. Tilt the slide to evenly coat its upper surface with fixative.
6. Immediately hold the slide over the water bath. Holding a Pasteur pipette 2 to 4 inches above the slide, expel 3 to 4 drops of cell specimen suspension along the length of the slide.
7. Place the slide, specimen side up, on the top of the test tube rack in the water bath. Let the slide dry for 10 minutes.
8. Remove the slide from the test tube rack and view under a phase contrast microscope. Examine the number of interphases per field, under low power (10X objective). A minimum of 100 cells per field is required for optimum assay results. Adjust the cell specimen suspension with fresh fixative to achieve the recommended number of interphase nuclei.
9. Gently outline the area containing the interphase nuclei on the back of the slide with a diamond-tipped scribe. Since a coverslip (22 mm × 22 mm) is to be used to form the hybridization zone, the area outlined should be within that of the coverslip. Up to 2 coverslips may be applied per slide.
10. Place the specimen slides in a slide box.
11. Age the specimen slides at room temperature for 24 hours, with the slide box uncovered, before hybridization or storage.

Slide Storage

Place the prepared slides in a covered slide box. Seal the slide box in a plastic bag purged with nitrogen gas and containing approximately 1 tablespoon of Drierite. Store at −20°C prior to hybridization.

ASSAY PROCEDURE: FISH PROCEDURE SUMMARY

Denaturation of Specimen DNA:

1. Prewarm the hybridization chamber (an airtight container) to 42°C by placing it in the 42°C incubator prior to slide preparation.
2. Add denaturing solution to Coplin jar and place in a 73±1°C water bath for at least 30 minutes. Verify the solution temperature before use.
3. Denature the specimen DNA by immersing the prepared slides in the denaturing solution at 73±1°C for 5 minutes. Do not denature more than 4 slides at 1 time per Coplin jar. Check that the pH of the denaturing solution is 7.0 to 8.0 before each use.
4. Using forceps, remove the slide(s) from the denaturing solution and immediately place into a 70% ethanol wash solution at room temperature for 1 minute. Agitate the slide to remove the formamide. Allow the slide(s) to stand in the ethanol wash for 1 minute.
5. Remove the slide(s) from 70% ethanol. Repeat step 4 with 85% ethanol, followed by 100% ethanol.
6. Drain the excess ethanol from the slide by touching the bottom edge of the slide to a blotter and wipe the underside of the slide dry with a laboratory wipe.
7. Place the slide(s) on a 45 to 50°C slide warmer no more than 2 minutes before you are ready to apply the probe solution.
   NOTE: If the timing of the hybridization is such that the slide is ready more than 2 minutes before the probe is ready, the slide should remain in the jar of 100% ethanol. Do not air-dry a slide before placing it on the slide warmer.

Probe Preparation

1. Allow the probe to warm to room temperature, thus decreasing the viscosity and allowing for accurate pipetting.
2. Vortex to mix. Spin the tubes briefly (1 to 3 seconds) in microcentrifuge to bring the contents to the bottom of the tube. Gently vortex again to mix.
   NOTE: The probe is predenatured and is ready to apply to the denatured target area on the specimen slide.

Hybridization

1. Apply the 10 µL aliquot of probe solution to the target area of the slide. Immediately, place a 22 mm × 22 mm glass coverslip over the probe solution and allow the solution to spread evenly under the coverslip. Air bubbles will interfere with hybridization and should be avoided.
   NOTE: Do not pipette probe solution onto multiple target areas before applying the coverslips.
2. Place the slide into the prewarmed 42°C hybridization chamber and cover the chamber with a tight lid.
3. Place the chamber containing the slide into the 42°C incubator and allow hybridization to proceed for at least 30 minutes.
   NOTE: Longer hybridization time may be required for sufficient signal intensity in some specimens. Incubations may be performed overnight (16 hours). For incubations longer than 1 hour, the coverslip must be sealed using a removable sealant such as rubber cement and the hybridization chamber must be humidified. The procedure is described below.

- Draw rubber cement into a 5 mL syringe. Exude a small amount of rubber cement around the periphery of the coverslip overlapping the coverslip and the slide, thereby forming a seal around the coverslip.
- Place the slide into a humidified hybridization chamber (an airtight container with a piece of damp blotting paper or paper towel approximately 1 in × 3 in taped to the side of the container).
- Cover the chamber with a tight lid and incubate 1 to 16 hours, as desired.
- Following incubation, remove the rubber cement from the coverslip by pulling up on the rubber cement.

Post-hybridization Washes

1. Add 0.4X SSC (pH 7.0 to 7.5) to a Coplin jar. Prewarm the 0.4X SSC solution by placing the Coplin jar in the 73±1°C water bath for at least 30 minutes or until the solution temperature has reached 73±1°C .
   NOTE: In order to maintain the proper temperature range, 4 slides MUST be placed in the heated wash solution at 1 time. If fewer than 4 slides have been hybridized, room temperature microscope slides (without specimen applied) may be used to bring the number of slides to 4. If more than 4 slides have been hybridized they must be washed in more than 1 batch. The temperature of the wash solution must return to 73±1°C before washing each batch.
2. Remove the coverslip from the target area of the first slide and immediately place the slide into the Coplin jar containing 0.4X SSC, 73±1°C. Agitate the slide for 1 to 3 seconds. Repeat for the other 3 slides and incubate for 2 minutes at 73±1°C.
   NOTE: Do not remove the coverslips from several slides before placing any of the slides in the wash bath. Begin timing the 2 minute incubation when the last slide has been added to the wash bath.
3. Remove each slide from the wash bath and place in the jar of 2X SSC/0.1% NP-40 at room temperature for 5 to 60 seconds, agitating for 1 to 3 seconds as the slides are placed in the bath.
4. Allow the slide to air-dry in the dark. (A closed drawer or a shelf inside a closed cabinet is sufficient.)
5. Apply 10 µL of DAPI II counterstain to the target area of the slide and apply a glass coverslip. Store the slide(s) in the dark prior to signal enumeration.

Storage

Store hybridized slides (with coverslips) at −20°C in the dark. Under these conditions the slides can be stored for up to 12 months without significant loss in fluorescence signal intensity. For long term storage, the coverslips may be sealed to prevent desiccation and the slides stored at −20°C.

Signal Enumeration

Assessing Slide Adequacy

Evaluate slide adequacy using the following criteria:

- Probe Signal Intensity: The signal should be bright, distinct, and easily evaluable. Signals should be in either bright, compact, oval shapes or stringy, diffuse, oval shapes.
- Background: The background should appear dark or black and free of fluorescence particles or haziness.
- Cross-hybridization/Target Specificity: The probe should hybridize and illuminate only the target (centromere of chromosome 8). Metaphase spreads should be evaluated to identify any cross-hybridization to nontarget sequences. At least 98% of cells should show 1 or more signals for acceptable hybridization (see guidelines for signal enumeration below).

If any of the above features are unsatisfactory, consult the troubleshooting guide, **Table 3**, and process a fresh slide.

Selection of Optimum Viewing Area and Evaluable Nuclei

Use a 10 to 25X objective to scan the hybridized area and examine the specimen distribution. Select an area where the specimen is distributed sparsely, few interphase nuclei or metaphase spreads are overlapping, and several interphase nuclei or metaphase spreads can be scanned within a viewing field. Avoid areas where the distribution of cells is dense, cells are overlapped, or the nuclear border of individual nuclei is unidentifiable. Avoid areas which contain clumps of cells. Enumerate only those cells with discrete signals.

Enumeration scan

Using a 40X or 63X objective, begin analysis in the upper left quadrant of the selected area and, scanning from left to right, count the number of signals in each evaluable metaphase spread or within the nuclear boundary of each evaluable interphase cell. Areas on the slide with a high cell density should be randomly skipped in order to scan the entire target area. Continue the scanning until 500 interphase nuclei are enumerated and a minimum of 20 metaphase spreads are counted and analyzed. If greater than 2% of the nuclei show no hybridization signal after enumerating 200 nuclei, the slide should be designated as a hybridization failure and no results should be reported.

Interphase Enumeration

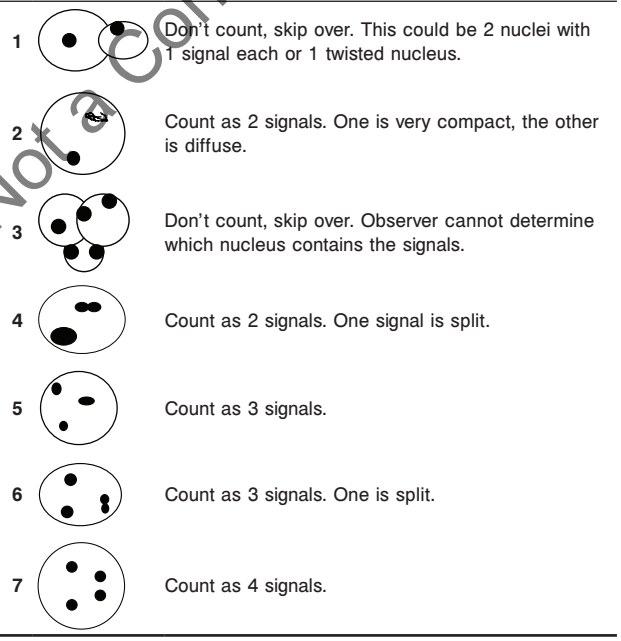
Enumerate the fluorescent signals in each evaluable interphase nucleus using a 40X or 63X objective. Follow the signal counting guidelines in the **Figure**. Objectives with higher magnification (eg, 63X or 100X) should be used to verify or resolve questions about split or diffused signals.

- Two signals that are in close proximity and approximately the same sizes but not connected by a visible link are counted as 2 signals.
- Count a diffuse signal as 1 signal if diffusion of the signal is contiguous and within an acceptable boundary.
- Two small signals connected by a visible link are counted as 1 signal.
- Enumerate the number of nuclei with 0, 1, 2, 3, 4, or >4 signals. Count nuclei with zero signals only if there are other nuclei with at least 1 signal present in the field of view. If the accuracy of enumeration is in doubt, repeat the enumeration in another area of the slide.
- Do not enumerate nuclei with uncertain signals.

Metaphase Enumeration

- The metaphase spread should have chromosomes that are well separated from each other but are clearly from the same cell. Select good quality, complete metaphase spreads with well defined, nonoverlapping chromosomes for chromosome enumeration and analysis.
- The CEP 8 DNA Probe signal will be visible as a distinct fluorescent signal located near the centromere region of the 8th chromosomes. The CEP signal may appear split (2 smaller signals in close proximity) if the chromatids are separated. Chromatid separation occurs when the cell is in the later stages of mitosis (between metaphase and anaphase). The split signal found on each of the 2 chromatids should be counted as 1 signal. Follow general magnification and scanning guidelines as indicated above in the Interphase Enumeration.

Figure. Image Examples and Interphase Signal Counting Guidelines



Quality Control

Use of Control Slides

Positive Control (low level trisomy 8) and Negative Control (approximately 0% trisomy 8) slides must be run concurrently with patient slides to monitor assay performance and to assess the accuracy of signal enumeration. Controls should be run on each day of FISH testing and with each new probe kit lot. The ProbeChek packages are available separately in addition to being available within a kit, the separate packages contain 5 positive slides (low level trisomy 8, Part No. 30-805002) or 5 negative slides (nontrisomy, Part No. 30-805000). The acceptable range for the % tri-signaled nuclei is indicated on the specification data sheet that accompanies these slides.

Slide adequacy and signal enumeration should be assessed using the criteria described above in the signal enumeration section. The criteria for slide adequacy must be satisfied and the signal enumeration results should be within the specifications on the data sheets provided with the control slides for acceptable test performance.

If control slides fail to meet the slide acceptance criteria, the assay may not have been performed properly or the CEP 8 SpectrumOrange DNA Probe Kit component(s) may have performed inadequately. A repeat analysis with fresh control slides and patient specimen slide(s) may be

necessary. Consult the troubleshooting guide in **Table 3** for probable causes and the actions needed to correct the problems.

If control slides meet the acceptance criteria but the enumeration values are outside the specified range, the enumeration may not have been performed correctly and an independent, repeat assessment of the same slide may be appropriate.

In no case should routine FISH test results be reported if assay controls fail. For clinical specimens, when interpretation of the hybridization signal is difficult and there is insufficient specimen sample for reassay, the test is uninformative. If there are insufficient cells for analysis, the test is uninformative.

Table 3. Troubleshooting Guide

Problem	Probable Cause	Solution
• No signal or weak signals	• Inappropriate filter set used to view slides	• Use correct filters
	• Microscope not functioning properly	• Call microscope manufacturer's technical representative
	• Improper lamps (ie, xenon or tungsten)	• Use a mercury lamp (100-watt recommended)
	• Mercury lamp too old	• Replace with a new lamp
	• Mercury lamp misaligned	• Realign lamp
	• Dirty and/or cracked collector lenses	• Clean and replace lens
	• Dirty or broken mirror in lamp housing	• Clean or replace mirror
	• Inappropriate objectives	• Use recommended objectives
	• Probes not denatured properly	• Check temperature of 73±1°C water bath
	• Hybridization conditions inappropriate	• Check temperature of 42°C incubator
		• Increase hybridization time to 1 hour
	• Wash conditions inappropriate	• Check temperature of 73±1°C water bath
		• Check formulation of wash baths (eg, pH)
	• Air bubbles trapped under coverslip and prevented probe access	• Apply coverslip by first touching the surface of the hybridization mixture
	• Probes improperly stored	• Store probes at −20°C in darkness
• Low signal specificity	• Hybridization conditions inappropriate	• Check temperature of 42°C incubator
	• Wash temperature too low	• Maintain wash temperature at 73±1°C
• High slide background	• Metaphase spreads were aged by baking or contain a lot of cytoplasm	• Increase slide denaturation time to 10 minutes
	• Cellular debris in cell preparation	• Wash cell preparation 5 times with fresh fixative and repeat **Slide Preparation** procedure
	• Specimen DNA not clean	• Replace 0.4X SSC post-hybridization wash with formamide wash as follows 1. wash slide(s) 3X for 10 minutes each in 50% formamide/2X SSC pH 7.5 to 8.0 at 46±1°C 2. wash slide(s) 1X for 10 minutes in 2X SSC at 46±1°C 3. wash slide(s) 1X for 5 minutes in 2X SSC/0.1% NP-40 at 46±1°C
	• Use of long-pass filters which pass a lot of light	• Switch to filters with smaller bandwidths or a multi-bandpass filter
	• Washes at wrong temperature or wrong formulation	• Check bath temperature, pH, and/or formulation
	• Slides not properly cleaned prior to dropping target cells onto them	• Dip slides in ethanol and wipe with laboratory wipe prior to dropping cells
• "Distorted" chromosome morphology	• Slides dried too quickly during sample preparation	• Increase relative humidity during sample slide preparation
		• Increase temperature of water bath during sample slide preparation
	• Slides too fresh prior to denaturation	• Increase sample slide drying time
		• Age slides at least 24 hours at room temperature prior to denaturation
	• Slides not thoroughly dry prior to denaturation	• Warm slides at 45°C for 10 to 15 minutes prior to denaturation
	• Temperature too high in denaturing bath	• Check water bath temperature
• Excessively bright signal	• Probe concentration too high for your microscope	• Try to block some of the signal by placing a neutral density filter in the excitation pathway

TEST INTERPRETATION

Results on enumeration of 500 interphase nuclei are reported as the number and percentage of nuclei with 1, 2, 3, 4, and >4 signals. Clinical specimens with >2.2% tri-signaled nuclei are considered to have an abnormal trisomy 8 clone; those with ≤2.2% are considered normal, although the presence of trisomy 8 can not be completely excluded. When the percentage of tri-signaled interphase nuclei are near the cutoff point (2.0 to 2.5%), the results should be interpreted with caution.

Results on enumeration of 20 to 30 metaphase spreads are reported as the number and percentage of metaphases with 1, 2, 3, 4, and >4 signals. Interpretation of the results follows the same rules as the standard cytogenetic analysis. Under these rules, a specimen is negative for trisomy 8 if no tri-signaled metaphases are found in 20 metaphases and positive for trisomy 8 if ≥2 tri-signaled metaphases are found in 20 to 30 metaphases. If 1 tri-signaled metaphase is found in 20 metaphases, an additional 10 metaphases are enumerated. If only 1 trisomy 8 is found in 30 metaphases, the specimen is ambiguous.

If standard cytogenetic and FISH results do not agree or the results are not consistent with the clinical findings, the basis for any discrepancies should be carefully examined. Discrepancies in results among test methods may be due to inaccurate results from 1 or more of the test methods, differences in analytic sensitivity/specificity among the methods, actual differences in chromosome 8 status among the cell populations assessed with the different methods (eg, cycling metaphase cells vs noncycling interphase cells), among others. When results of 1 or more test method are ambiguous, or when the percentage of tri-signaled interphase nuclei are near the cutoff point (2.0 to 2.5%), the results should be interpreted with caution and further assessment of the test specimen may be required. Repeat FISH (run concurrently with QC material) and/or repeat standard cytogenetic analysis with the remaining sample may be useful to assess the possibility of incorrect test results. If the basis for any discrepancies in the test results is not determined or if the test results are not consistent with the clinical findings, a consultation between the cytogeneticist and the treating physician is warranted.

LIMITATIONS

1. The CEP 8 SpectrumOrange DNA Probe Kit has been characterized only for identifying chromosomes in nuclear preparations or metaphase spreads from bone marrow specimens.
2. The clinical interpretation of any test results should be done in conjunction with standard cytogenetic analysis and should be evaluated within the context of the patient's medical history and other diagnostic laboratory test results.
3. Clinical specimens with >2.2% tri-signaled nuclei are considered to have an abnormal trisomy 8 clone. Those with ≤2.2% tri-signaled nuclei should be considered normal, although the presence of trisomy 8 is not completely excluded.
4. The CEP 8 SpectrumOrange DNA Probe Kit is not intended for long term cell cultured materials such as amniocytes, fibroblasts, and tumor cells.
5. FISH assay results may not be informative if the specimen quality and/or specimen slide preparation is inadequate.
6. If significant peripheral blood contamination is present in the bone marrow specimen, the blood may dilute the specimen; it is important to recognize the potential effects this dilution effect may have on the FISH assay results.
7. It is possible that patients may have chromosome polymorphism which may hybridize with CEP 8 probe. FISH metaphase analysis should be done in addition to FISH interphase analysis. Polymorphism was not investigated in the clinical trials.
8. This assay will not detect the presence of other chromosome abnormalities frequently associated with hematological disorders.
9. The efficacy of this assay for monitoring of trisomy 8 or disease progression has not been demonstrated.

EXPECTED VALUES

FISH interphase signal enumeration was performed on bone marrow specimens obtained from normal subjects and from subjects positive for trisomy 8 to assess the expected percentage of cells with 0, 1, 2, 3, and ≥4 signals for these 2 states and to determine the cutoff for defining the presence or absence of trisomy 8. Each specimen was enumerated for the percentage of cells with 0, 1, 2, 3, and ≥4 signals.

Values Among Normal Bone Marrow Specimens

FISH interphase analysis was initially performed on bone marrow specimens from 35 normal subjects. The signal distribution for this study population is summarized in **Table 4**.

Table 4. Distribution of Percentage of Cells with CEP 8 Probe Signals in 35 Normal Bone Marrow Specimens

	Percent of Cells With				
	0 signal	**1 signal**	**2 signals**	**3 signals**	**≥4 signals**
Mean	0.22	2.23	96.7	0.71	0.18
SD	0.20	0.91	0.88	0.52	0.13

In addition, a pivotal study was performed on 60 cytogenetically normal bone marrow specimens, collected at 3 sites, to confirm the expected values and to determine the cutoff of the assay. The distribution of signals for these 60 subjects is summarized in **Table 5**.

Table 5. Distribution of Percentage of Cells with CEP 8 Probe Signals in 60 Normal Bone Marrow Specimens

	Percent of Cells With				
	0 signal	**1 signal**	**2 signals**	**3 signals**	**≥4 signals**
Mean	0.11	2.67	96.7	0.41	0.14
SD	0.21	1.95	2.05	0.24	0.20

In cytogenetically normal bone marrow specimens, the percentage of bi-signaled and tri-signaled cells are 2 critical categories for assessing expected values. The average percentages of bi-signaled and tri-signaled nuclei in the initial study were 96.7% (SD = 0.88%) and 0.71% (SD = 0.52%), respectively. Similarly, in the confirmatory study, the average percentages of bi-signaled and tri-signaled nuclei for the 60 normal specimens were 96.7% (SD = 2.05%) and 0.41% (SD = 0.24%), respectively. Thus, when the recommended enumeration guide is followed and practiced, the percent of bi-signaled cells in a normal bone marrow specimen should be between 92.7% and 100% (95% CI) and the percent of tri-signaled cells should be between 0% and 1.73% (95% CI).

Values Among Bone Marrow Specimens Positive for Trisomy 8

A study was performed to assess the distribution of interphase FISH signals in 151 bone marrow specimens previously determined to be positive for trisomy 8 by standard cytogenetic analysis. The distribution of signals for these 151 subjects is summarized in **Table 6**.

Table 6. Distribution of Percentage of Cells With CEP 8 Probe Signals in 151 Bone Marrow Specimens with Trisomy 8

	Percent of Cells With				
	0 signal	**1 signal**	**2 signals**	**3 signals**	**≥4 signals**
Mean	0.08	1.21	48.76	48.81	1.22
SD	0.22	1.22	28.90	29.58	6.42

In cytogenetically abnormal bone marrow specimens, the critical category is the percentage of cells with 3 signals (% tri-signaled nuclei). Based on FISH analysis of the 151 specimens, the mean (±SD) % tri-signaled nuclei was 48.81% (±29.58%). The % tri-signaled nuclei ranged from 0.6% to 96.6%.

Determination of the Cutoff

The other critical category in normal bone marrow specimens is the percent tri-signaled nuclei. The percent tri-signaled nuclei in normal specimens was used for the determination of a cutoff point for trisomy 8. The percent of tri-signaled cells was calculated for each of the 60 subjects in the pivotal study and the data distribution was assessed for normal distribution using the Shapiro-Wilk W Test.[10] The normal assumption was not satisfied and the data were log-transformed and the mean percentage of tri-signaled cells and the standard deviation was calculated. The 95 percent confidence limits were determined using a normal approximation and the antilog value of the upper limit was used as the cutoff. Details of the calculation are shown below in **Table 7**.

Table 7. Determination of Cutoff Point for Classifying Trisomy 8

	Percent Tri-signaled Nuclei	
Statistics	**Log-transformed Values**	**Antilog Arithmetic Value**
Mean	−0.4783	—
Standard Deviation	0.4178	—
Upper 95% Confidence Interval Mean + 1.96 SD	0.3406	2.19

The value for the cutoff point, 2.19%, calculated in **Table 7** was rounded to 2.2%. Normal (negative) values are defined as ≤2.2 tri-signaled cells, while >2.2% tri-signaled cells are defined as positive for the presence of trisomy 8.

A cutoff of 2.2% is also supported by receiver operator characteristic (ROC) curve analysis for maximizing both the relative sensitivity and specificity.

Prior to using the CEP 8 kit, the laboratory should verify its cutoff by analyzing and enumerating a minimum of 10 bone marrow specimens according to the instructions in the enumeration section of the package insert. The percent tri-signaled nuclei in these normal specimens should all be below the cutoff point of 2.2%. If this cutoff point is not appropriate for the user's institution, the user may choose to redefine this cutoff point by following the statistical procedure described above. Note that a sample size of 10 will not suffice for defining a new cutoff.

SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Sensitivity and Specificity

Hybridization Efficiency

In a pilot study, the average percentage of cells with no hybridization signal was 0.22% (SD = 0.20%) on 35 bone marrow (BM) specimens. In a pivotal study, the average percentage of cells with no hybridization signal was 0.11% (SD = 0.21%) on 60 BM specimens. Thus, < 2% cells with no signal is a realistic standard of acceptance, especially for FISH metaphase analysis.

Analytical Sensitivity

The analytical sensitivity of the CEP 8 probe was tested in the reproducibility study described below. In that study, the 0% specimen was estimated with a mean of 0.95% (SD = 0.51%) tri-signaled nuclei and the 5% specimen, 5.37% (SD = 0.98%). There was little overlap between the 0% and 5% specimens; the upper 95% confidence limit for the 0% specimen was 1.95% and the lower 95% confidence limit for the 5% specimen was 3.45%. Thus, the limit of detection for CEP 8 in interphase cells is estimated to be 4.0%.

Analytical Specificity

Locus-specificity studies were performed with metaphase spreads according to standard Abbott Molecular QC protocols. A total of 62 metaphase spreads were examined sequentially by G-banding to identify chromosome 8, followed by FISH. No cross-hybridization to other chromosome loci was observed in any of the 62 cells examined; hybridization was limited to the centromere region of chromosome 8.

Reproducibility

A pilot study was conducted to assess the reproducibility of CEP 8 interphase analysis for the percentage of tri-signaled cells, using one lot of the CEP 8 DNA probe. Bone marrow specimen slides from a normal donor were prepared in 1 internal site and distributed to 2 sites for FISH assay and signal enumeration performed by 4 observers on different days. The mean, standard deviation, and percent CV of the observed percentage of tri-signaled nuclei are shown in **Tables 8** through **12**.

To further assess the reproducibility of the CEP 8 assay, CEP 8 analyses for the percentage of tri-signaled cells were assessed for intersite, interlot, interday, and interobserver reproducibility. One low level (approximately 7%) trisomy 8 bone marrow specimen was evaluated for the percentage of tri-signaled cells according to the instructions for signal enumeration in the package insert. The mean, SD, and percent CV of the observed percentage of tri-signaled nuclei for each variable are shown in **Tables 8** through **12**.

Table 8. Precision of the Observed % Tri-signaled Nuclei

Specimen Level of Trisomy 8	n	Mean	Standard Deviation	Coefficient of Variation
0%	24	0.99%	0.57%	58%
7%	24	7.70%	1.45%	19%

Table 9. Summary Statistics of % Tri-signaled Nuclei by Study Site

Level of Trisomy 8	Statistics	Site #1	Site #2	Site #3
0%	Mean	0.83	1.15	—
	SD	0.40	0.67	—
	CV(%)	48.20	46.20	—
	n	12.00	12.00	—
7%	Mean	8.93	8.02	6.17
	SD	0.96	1.09	1.10
	CV(%)	10.70	13.60	17.8
	n	8.00	8.00	8.00

SD (Standard Deviation), CV(%) (Coefficient of Variation)

Table 10. Summary Statistics of % Tri-signaled Nuclei by Probe-Lot

Level of Trisomy 8	Statistics	Lot #1	Lot #2	Lot #3	Lot #4
0%	Mean	0.99	—	—	—
	SD	0.57	—	—	—
	CV(%)	58.00	—	—	—
	n	24.00	—	—	—
7%	Mean	8.30	7.63	7.80	7.10
	SD	1.72	1.45	0.95	2.02
	CV(%)	20.70	19.00	12.10	28.50
	n	6.00	6.00	6.00	6.00

SD (Standard Deviation), CV(%) (Coefficient of Variation)

Table 11. Summary Statistics of % Tri-signaled Nuclei by Assay Day

Level of Trisomy 8	Statistics	Assay Day #1	Assay Day #2	Assay Day #3	Assay Day #4
0%	Mean	0.83	1.15	—	—
	SD	0.40	0.67	—	—
	CV(%)	48.20	46.20	—	—
	n	12.00	12.00	—	—
7%	Mean	8.23	7.67	7.17	7.76
	SD	1.70	1.40	2.20	0.73
	CV(%)	20.70	18.30	30.70	9.40
	n	6.00	6.00	6.00	6.00

SD (Standard Deviation), CV(%) (Coefficient of Variation)

Table 12. Summary Statistics of % Tri-signaled Nuclei by Observer

Level of Trisomy 8	Statistics	Observer #1	Observer #2
0%	Mean	0.75	1.23
	SD	0.64	0.37
	CV(%)	85.30	30.10
	n	12.00	12.00
7%	Mean	7.82	7.60
	SD	1.35	1.76
	CV(%)	17.30	23.20
	n	12.00	12.00

SD (Standard Deviation), CV(%) (Coefficient of Variation)

Significant site-to-site and observer-to-observer variations were observed, reflecting the subjectivity of the visual enumeration process. The results of classification of slides as positive or negative for trisomy 8 (using a cutoff of 2.2%) was 96% correct (1 of 24 had 2.4% tri-signaled nuclei) for the normal bone marrow specimen and 100% correct for the low level (7%) trisomy 8 specimen.

Methods Comparison; Clinical Specimens

A multicenter, blinded, controlled, comparative study was conducted to further define the performance characteristics of the CEP 8 SpectrumOrange DNA probe kit. The objective of the study was to determine the sensitivity and specificity of the CEP 8 assay relative to standard cytogenetic analysis, the standard of care. Four laboratories provided 368 archived bone marrow specimens for assay at 3 investigation sites. Site 1 provided 101 specimens; Site 2 provided 57 specimens; Site 3 provided 130 specimens; Site 4 provided 80 specimens. Specimens from Site 4 were analyzed at Site 3. By standard cytogenetic analysis, 151 of these specimens were classified as positive for trisomy 8; 201 negative for trisomy 8; and 16 ambiguous for trisomy 8 (1 trisomy 8 per 30 metaphases analyzed). Fifteen of the 16 ambiguous cases were selected “purposefully” after study completion. These specimens were derived from patients with 1 of the following diagnoses.

1. Acute myeloid leukemia **(AML)**: 102 specimens
2. Myeloproliferative disorder **(MPD)**, including polycythemia vera: 44 specimens
3. Myelodysplastic syndrome **(MDS)**: 80 specimens
4. Chronic myelogenous leukemia **(CML)**: 72 specimens

5. Hematological disorder, not otherwise specified **(HDNOS)**: 70 specimens (including hyperproliferative states such as leukemoid reaction, lymphoproliferative disorders or chronic lymphocytic leukemia, without trisomy 8).

At one trial site, approximately 50% of the archived bone marrow specimens failed to produce informative FISH results. Further examination of a subset of these specimens revealed a lack of specimen integrity and it was determined that specimens at this site were stored at 4°C rather than at the recommended temperature of −20°C. The conclusion was that some specimens and/or slide preparations were inadequate.

FISH Interphase Analysis versus Standard Cytogenetics

From the same multicenter comparative study described above, the analysis of interphase nuclei by FISH compared to standard cytogenetics was performed. Based on the cutoff point of 2.2% tri-signaled nuclei that was validated by the same pivotal clinical study, the relative sensitivity was 96.03% (145/151) [95% CI 92.55 to 99.51%] and the relative specificity was 98.01% (197/201) [95% CI 96.08 to 99.94%] for the CEP 8 interphase analysis. The results are shown in **Table 13**.

Among the 10 discrepant cases, the standard cytogenetic analysis results ranged from 0/20 to 8/22 metaphase cells with trisomy 8; the CEP 8 interphase results of % tri-signaled nuclei ranged from 0.6% to 6.0%. Among the 16 ambiguous cases, the range of % tri-signaled nuclei by CEP 8 interphase analysis was from 0.2% to 2.2%.

Table 13. Relative to Standard Cytogenetics, the Sensitivity and Specificity of CEP 8 FISH Interphase Analysis at the Validated Cutoff Point (2.2%) All 4 Sites Combined

	Standard Cytogenetic Analysis			
CEP 8 Interphase Analysis	**Positive**	**Negative**	**Ambiguous**	**Total**
Positive	145 (96.03%)	4	0	149
Negative	6	197 (98.01%)	16	219
TOTAL	**151**	**201**	**16**	**368**

From the 368 clinical specimens, the correlation coefficient of trisomy 8 between standard cytogenetic metaphase analysis and CEP 8 interphase analysis was 0.91. The regression coefficient of CEP 8 assay on cytogenetic analysis was 0.71. The following equation describes the plot of CEP 8 assay results on cytogenetic analysis results:

$$y = 1.2944 + 0.7112x$$

where: x = percent metaphase spreads with trisomy 8 by standard cytogenetics

y = percent tri-signaled interphase nuclei by CEP 8 analysis

FISH Metaphase Analysis versus Standard Cytogenetics

A total of 348 cases were included in the comparison of CEP 8 metaphase analysis to standard cytogenetics. Twenty of the 368 cases included in the CEP 8 interphase analysis were excluded due to insufficient number of metaphase spreads for analysis with the CEP 8 assay (a specimen must have ≥ 20 metaphase spreads.)

By the rules of standard cytogenetic analysis, a case is declared positive for trisomy 8 if 2 or more metaphase spreads are trisomic for chromosome 8. Using this cutoff of ≥ 2 tri-signaled metaphases, the relative sensitivity was 89.19% (132/148) [95% CI 84.19 to 94.19%] and the relative specificity was 91.30% (168/184) [95% CI 87.22 to 95.37%] for the CEP 8 metaphase analysis. The results are shown in **Table 14**. Among the 16 ambiguous cases by standard cytogenetic analysis, 15 were negative and 1 was ambiguous for trisomy 8 by CEP 8 metaphase analysis.

Table 14. Relative to Standard Cytogenetics, the Sensitivity and Specificity of CEP 8 FISH Metaphase Analysis, Using a Cutoff Point of ≥ 2 Tri-signaled Metaphases

	Standard Cytogenetic Analysis			
CEP 8 Metaphase Analysis	**Positive**	**Negative**	**Ambiguous**	**Total**
Ambiguous	6	12	1	19
Positive	132 (89.19%)	4	0	136
Negative	10	168 (91.30%)	15	193
TOTAL	**148**	**184**	**16**	**348**

From the 348 clinical specimens, the correlation coefficient of trisomy 8 between cytogenetic metaphase analysis and CEP 8 metaphase analysis was 0.91. The regression coefficient of CEP 8 assay on cytogenetic analysis was 0.83. The following equation describes the plot of CEP 8 assay results on cytogenetic analysis results:

$$y = 1.6979 + 0.8325x$$

where: x = percent metaphase spreads with trisomy 8 by standard cytogenetics

y = percent tri-signaled metaphase spreads by CEP 8 analysis

FISH Interphase Analysis versus FISH Metaphase Analysis

In addition to the methods comparison between FISH and standard cytogenetics described above, a comparison between FISH interphase and FISH metaphase was made. **Table 15** shows that there is a 90.8% [(133 + 183)/348] concordance between them.

Table 15. Comparison of CEP 8 Interphase and CEP 8 Metaphase Analysis, Using a Cutoff Point of ≥ 2 Tri-signaled Metaphases

	CEP 8 Interphase Analysis (cutoff = 2.2%)		
CEP 8 Metaphase Analysis	**Positive**	**Negative**	**Total**
Ambiguous	5	14	19
Positive	133	3	136
Negative	10	183	193
TOTAL	**148**	**200**	**348**

From the 348 clinical specimens, the correlation coefficient of trisomy 8 between CEP 8 interphase analysis and CEP 8 metaphase analyses was 0.95. The regression coefficient of CEP 8 metaphase analysis on interphase analysis was 0.81. The following equation describes the plot of CEP 8 assay metaphase results on CEP 8 interphase analysis results:

$$y = 1.0942 + 0.8119x$$

where: x = percent tri-signaled interphase cells by CEP 8 analysis

y = percent tri-signaled metaphase spreads by CEP 8 analysis

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TECHNICAL ASSISTANCE

For technical assistance, call Abbott Molecular Technical Services at 1-800-553-7042 (within the US) or +49-6122-580 (outside the US), or visit the Abbott Molecular Web site at http://www.abbottmolecular.com.

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