

# CEP 12 SpectrumOrange DNA Probe Kit

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**REF** 07J20-012  
07J22-012

## CEP 12 SpectrumOrange DNA Probe Kit

**REF** 07J20-012; 07J22-012

30-608311/R2

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### Key to Symbols Used



Manufacturer



Reference Number



In Vitro Diagnostic Medical Device



Contains sufficient for <n> tests



Temperature Limitation



Danger



Danger



Danger



Biological Risks



Consult instructions for use



Used By



Authorized Representative in the European Community

## CEP 12 SPECTRUMORANGE DIRECT LABELED CHROMOSOME ENUMERATION DNA PROBE KIT FOR FLOURESCENCE IN SITU HYBRIDIZATION

(Part No. 30-160-012, List No. 07J20-012;

Part No. 30-220-012, List No. 07J22-012)

### PROPRIETARY NAME

CEP 12 SpectrumOrange Direct Labeled Fluorescent DNA Probe Kit

### COMMON OR USUAL NAME

Fluorescence in situ hybridization (FISH) reagents

### INTENDED USE

The CEP 12 SpectrumOrange DNA Probe Kit is intended to detect AT rich alpha satellite sequences in the centromere region of chromosome 12 in conjunction with routine diagnostic cytogenetic testing. It is indicated for use as an adjunct to standard cytogenetic analysis for identifying and enumerating chromosome 12 via fluorescence in situ hybridization (FISH) in interphase nuclei of cells obtained from peripheral blood lymphocytes in patients with B-cell chronic lymphocytic leukemia (CLL). It is not intended to be used as a stand alone assay for test reporting; FISH results are intended to be reported and interpreted

only in conjunction with results of standard cytogenetic analysis, performed concurrently, using the same patient specimen. The CEP 12 assay has not been validated for purposes other than those described above. It is not intended for use with test matrices other than peripheral blood lymphocytes from subjects with CLL, to screen for chromosome 12 aneuploidy, eg, in asymptomatic individuals, or to monitor patients for residual disease.

### SUMMARY AND EXPLANATION

Currently, most patients diagnosed with CLL have early-stage disease (Rai stage 0 or 1). Patients with early stage CLL are a heterogeneous group; approximately 30 to 50% are at high risk of accelerated disease progression, and the remainder may live for decades and possibly never require therapy. Recent insights into the biological characteristics of leukemic B cells have led to the discovery of new prognostic tools (immunoglobulin variable region heavy chain gene mutation status, cytogenetic abnormalities assessed by FISH, and Z-chain-associated protein kinase-70 protein expression) that can contribute to the identification of patients with early-stage disease who are at high risk for early disease progression.<sup>1</sup>

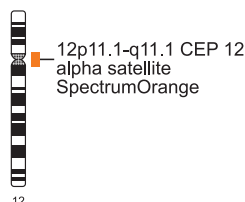
Routine karyotype analysis only detects chromosomal aberrations associated with CLL in 40 to 50% of the cases. Use of FISH and other technologies have detected genomic abnormalities in over 80% of cases of CLL. The common genomic aberrations seen are trisomy 12 and deletions of 13q, 17p, and 11q.<sup>2-4</sup> Traditional methodologies for measuring trisomy 12 in CLL have certain limitations in that, metaphase cells are required for analysis and there are relatively few leukemia cells available for analysis in CLL, since the in vitro mitotic rate of the leukemic cells is low. Therefore, results of traditional metaphase analyses may not reflect the actual proportions of cells with or without trisomy 12 in the specimen.<sup>6</sup> The FISH technique using probes to chromosome 12 centromeres may be used to assess chromosome 12 status in interphase cells.<sup>7-10</sup>

### 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.

Peripheral blood specimens consisting of interphase cells 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 12 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 12 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 12 and the blue counterstained chromosome and nuclei. The enumeration of chromosome 12 is conducted by microscopic examination of interphase nuclei. The fluorescently-stained centromeres of chromosome 12 stand out brightly against the general blue fluorescence of the nuclear DNA provided by the DAPI counterstain. The CEP 12 procedure enables visual enumeration of chromosome 12 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 12. 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 12 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 12 (12p11.1-q11.1). This assay is designed for the detection and quantification of chromosome 12 in interphase nuclei 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 12 SpectrumOrange DNA Probe Kit**  
(Part No. 30-160012, List No. 07J20-012)

Component	Composition	Part No.	Contents	Storage
CEP 12 DNA Probe: E. coli plasmid (The probe is predenatured)	2 ng/μL SpectrumOrange fluorophore-labeled alpha satellite DNA probe premixed with Blocking DNA and Hybridization Buffer. (dextran sulfate, formamide, SSC)	30-170012	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 12 SpectrumOrange DNA Probe Kit with ProbeChek Control Slides**  
(Part No. 32-160012, List No. 07J22-012.)

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	30-105000	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 unopened CEP 12 DNA probe kit as a unit at –20°C protected from light and humidity. The 20X SSC salts and NP-40 may be stored separately at room temperature. Store the ProbeChek Control Slides at –20°C in a sealed container with desiccant to protect them from humidity. Expiration dates for each of the unopened kit components are indicated on the individual component labels. These storage conditions apply to both opened and unopened components.

## 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

### 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 (3). Suggested type: Wheaton Product. No. 900620 vertical staining jar
- pH meter and pH paper
- Calibrated thermometer
- Wire test tube racks
- Rubber cement
- 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 autofluorescence 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 12 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) (see Abbott Molecular price list or 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

- For In Vitro Diagnostic Use
- 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.<sup>11</sup>
- Hybridization conditions may be adversely affected by the use of reagents other than those provided or recommended by Abbott Molecular, Inc.
- Failure to follow all procedures for slide denaturation, hybridization, and signal detection may cause unacceptable or erroneous results.
- 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.
- CEP 12 Probe contains formamide, a teratogen. Avoid contact with skin and mucous membranes.
- Calibrated thermometers are required for measuring temperatures of solutions, water baths, and incubators.
- All hazardous materials should be disposed of according to your local and state guidelines for hazardous disposal.

## CEP 12 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,<sup>13</sup> OSHA Standards on Bloodborne Pathogens,<sup>14</sup> CLSI Document M29-A3,<sup>15</sup> and other appropriate biosafety practices.<sup>16</sup> Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.<sup>13</sup>
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.<sup>16</sup>

## CEP 12 SpectrumOrange DNA Probe



### 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.
- P231 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

Peripheral blood collections should be performed according to the laboratory's institution guidelines. The ACT Manual contains a number of recommendations for collection and culturing of specimens.<sup>5</sup> Specimen preparation and culture conditions should be appropriate for B-cell analysis, using tumor promoting agent (TPA) and/or interleukin-2 (IL-2) as mitogens. According to the ACT Manual, blood can be cultured up to 4 days after collection, although care must be taken to avoid extremes in temperature. Specimens should be stored at room temperature or refrigerated above 4°C; blood specimens should never be iced or frozen.<sup>5</sup>

Icteric or hemolyzed specimens may prevent proper culture. 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  $67 \pm 2^\circ\text{C}$ . 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  $\times$  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  $-20^\circ\text{C}$  prior to hybridization.

#### **ASSAY PROCEDURE: FISH PROCEDURE SUMMARY**

##### Denaturation of Specimen DNA:

1. Prewarm the hybridization chamber (an airtight container) to  $42^\circ\text{C}$  by placing it in the  $42^\circ\text{C}$  incubator prior to slide preparation.
2. Add denaturing solution to Coplin jar and place in a  $73 \pm 1^\circ\text{C}$  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  $73 \pm 1^\circ\text{C}$  for 5 minutes. Do not denature more than 4 slides at a 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. 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  $50^\circ\text{C}$  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  $\mu\text{L}$  aliquot of probe solution to the target area of the slide. Immediately, place a 22 mm  $\times$  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  $42^\circ\text{C}$  hybridization chamber and cover the chamber with a tight lid.
3. Place the chamber containing the slide into the  $42^\circ\text{C}$  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  $\times$  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  $73 \pm 1^\circ\text{C}$  water bath for at least 30 minutes or until the solution temperature has reached  $73 \pm 1^\circ\text{C}$ .

**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  $73 \pm 1^\circ\text{C}$  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,  $73 \pm 1^\circ\text{C}$ . Agitate the slide for 1 to 3 seconds. Repeat for the other 3 slides and incubate for 2 minutes at  $73 \pm 1^\circ\text{C}$ .

**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  $\mu\text{L}$  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  $-20^\circ\text{C}$  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 should be sealed to prevent desiccation and the slides stored at  $-20^\circ\text{C}$ .

##### 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 12). Metaphase spreads should be evaluated to verify locus specificity and 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 25X objective to scan the hybridized area and examine the specimen distribution. Select an area where the specimen is distributed sparsely, few interphase nuclei are overlapping, and several interphase nuclei 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

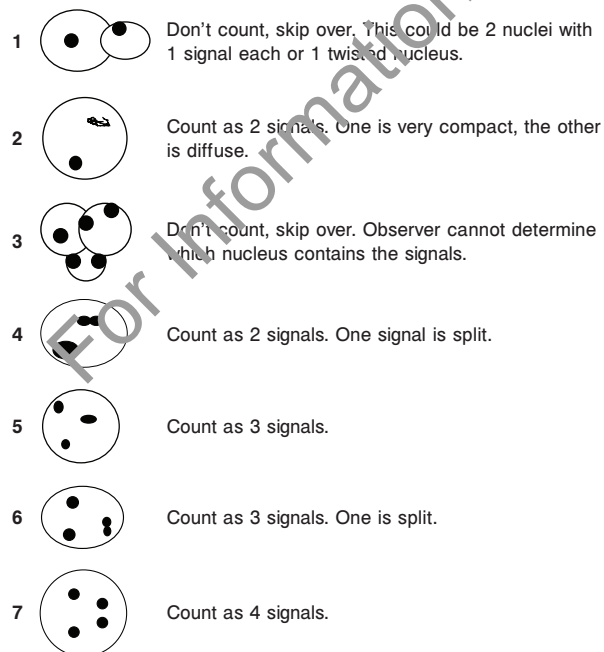
Using a 40X or 63X objective, begin analysis in the upper left quadrant of the selected area and, scanning from left to right, count the number of signals in each evaluable metaphase spread or within the nuclear boundary of each evaluable interphase cell. Areas on the slide with a high cell density should be randomly skipped in order to scan the entire target area. Continue the scanning until 500 interphase nuclei are enumerated and 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 0 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.

**Figure.** Image Examples and Interphase Signal Counting Guidelines



## Quality Control

### Use of Control Slides

Positive control (low-level trisomy 12) and negative control (approximately 0% trisomy 12) 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. ProbeChek slides are recommended.

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 12 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 problem.

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 re-assay, the test is uninformative. If there are insufficient cells for analysis, the test is uninformative.

Metaphase spreads should be analyzed to verify locus specificity and to assure that there are no cross-hybridization sequences at alternate chromosome locations.

**NOTE: The ProbeChek packages contain 5 positive slides (low-level trisomy 8/12, Part No. 30-805002) or 5 negative slides (non trisomy, Part No. 30-805000). The acceptable range for the % tri-sigaled interphase nuclei is indicated on the specification data sheet that accompanies these slides.**

**Table 3.** Troubleshooting Guide

Problem	Probable Cause	Solution
• No signal or weak signals	<ul style="list-style-type: none"> <li>• Inappropriate filter set used to view slides</li> <li>• Microscope not functioning properly</li> <li>• Improper lamps (ie, xenon or tungsten)</li> <li>• Mercury lamp too old</li> <li>• Mercury lamp misaligned</li> <li>• Dirty and/or cracked collector lenses</li> <li>• Dirty or broken mirror in lamp housing</li> <li>• Inappropriate objectives</li> <li>• Probes not denatured properly</li> <li>• Hybridization conditions inappropriate</li> <li>• Wash conditions inappropriate</li> <li>• Air bubbles trapped under coverslip and prevented probe access</li> <li>• Probes improperly stored</li> </ul>	<ul style="list-style-type: none"> <li>• Use correct filters</li> <li>• Call microscope manufacturer's technical representative</li> <li>• Use a mercury lamp (100-watt recommended)</li> <li>• Replace with a new lamp</li> <li>• Realign lamp</li> <li>• Clean and replace lens</li> <li>• Clean or replace mirror</li> <li>• Use recommended objectives</li> <li>• Check temperature of 75±1°C water bath</li> <li>• Check temperature of 42°C incubator</li> <li>• Increase hybridization time to 1 hour</li> <li>• Check temperature of 75±1°C water bath</li> <li>• Check formulation of wash baths (eg, pH)</li> <li>• Apply coverslip by first touching the surface of the hybridization mixture</li> <li>• Store probes at -20°C in darkness</li> </ul>
• Low signal specificity	<ul style="list-style-type: none"> <li>• Hybridization conditions inappropriate</li> <li>• Wash temperature too low</li> </ul>	<ul style="list-style-type: none"> <li>• Check temperature of 42°C incubator</li> <li>• Maintain wash temperature at 75±1°C</li> </ul>

**Table 3. Troubleshooting Guide (Continued)**

Problem	Probable Cause	Solution
• High slide background	• Cellular debris in cell preparation	• Wash cell preparation 5 times with fresh fixative and repeat <b>Slide Preparation</b> procedure
	• Specimen DNA not clean	• Replace 0.4X SSC post-hybridization wash with formamide wash as follows <ol style="list-style-type: none"> <li>1. wash slide(s) 3X for 10 minutes each in 50% formamide/2X SSC pH 7.5 to 8.0 at 46±1°C</li> <li>2. wash slide(s) 1X for 10 minutes in 2X SSC at 46±1°C</li> <li>3. wash slide(s) 1X for 5 minutes in 2X SSC/0.1% NP-40 at 46±1°C</li> </ol>
• “Distorted” chromosome morphology	• 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
	• Slides dried too quickly during sample preparation	• Increase relative humidity during sample slide preparation
• Excessively bright signal	• Slides too fresh prior to denaturation	• Increase temperature of water bath during sample slide preparation
	• Slides not thoroughly dry prior to denaturation	• Increase sample slide drying time
	• Temperature too high in denaturing bath	• Age slides at least 24 hours at room temperature prior to denaturation
	• Probe concentration too high for your microscope	• Warm slides at 45°C for 10 to 15 minutes prior to denaturation
		• Check water bath temperature
		• 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 >2.0% tri-signal nuclei are considered to have an abnormal trisomy 12 clone; those with ≤2.0% are considered normal, although the presence of trisomy 12 can not be completely excluded. When the percentage of tri-signal interphase nuclei are near the cutoff point (1.5 to 2.5%), the results should be interpreted with caution. 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 12 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-signal interphase nuclei are near the cutoff point (1.5 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 12 DNA Probe Kit has been optimized only for identifying chromosome 12 in interphase nuclei from peripheral blood specimens from patients with B-cell chronic lymphocytic leukemia.
2. The clinical interpretation of any abnormality or its absence by FISH should be done in conjunction with standard cytogenetic analysis and proper controls, and should be evaluated within the context of the patient's medical history and past diagnostic laboratory test results.
3. Clinical specimens with >2.0% tri-signal nuclei are considered to have an abnormal trisomy 12 clone. Those with ≤2.0% tri-signal nuclei should be considered normal, although the presence of trisomy 12 is not completely excluded. When the percentage of tri-signal interphase nuclei are near the cutoff point (1.5 to 2.5%), the results should be interpreted with caution.
4. The CEP 12 assay has been validated only for use with peripheral blood lymphocytes obtained from patients with B-cell CLL leukemia. It is not intended to be used for chromosome 12 enumeration in other patient populations or with other test matrices such as amniocytes, chorionic villi, fibroblasts, tumor cells, long term cultures, among others.
5. FISH assay results may not be informative if the specimen quality and/or specimen slide preparation is inadequate; the CEP 12 DNA Probe Kit has been optimized for archived peripheral blood specimens stored only at -20°C or -80°C, as recommended.
6. The CEP 12 DNA Probe Kit has not been validated for monitoring disease status.
7. It is possible that some individuals may have target sequences at an alternate chromosomal location that may hybridize with CEP 12. This has not been investigated for this device; the user should assess this in metaphase spreads from each subject tested.
8. The CEP 12 assay is intended only to aid in the enumeration of chromosome 12 centromeres; it is not designed to detect chromosome 12 structural abnormalities.

**EXPECTED VALUES**

FISH interphase signal enumeration was performed on peripheral blood specimens obtained from normal subjects and from subjects positive for trisomy 12 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 12.

**Values Among Normal Peripheral Blood Specimens**

FISH interphase analysis was performed on peripheral blood specimens from 30 normal subjects, at 1 pilot study site. Each specimen was enumerated for the percentage of cells with 0, 1, 2, 3, and ≥4 signals. The signal distribution for this study population is summarized in **Table 4**.

**Table 4. Distribution of Percentage of Cells with CEP 12 Signals in 30 Normal Peripheral Blood Specimens**

	Percent of Cells With				
	0 signal <sup>a</sup>	1 signal	2 signals	3 signals	≥4 signals
Mean	—	2.24	97.39	0.36	0.01
SD	—	1.29	1.29	0.26	0.03

<sup>a</sup> Data not collected

In addition, a pivotal study was performed on peripheral blood specimens from 57 (17 cultured and 40 uncultured) normal subjects, to confirm the expected values and to determine the cutoff of the assay. The sample collection and analysis was performed at 2 study sites. Each specimen was enumerated for the percentage of cells with 0, 1, 2, 3, and ≥4 signals. The distribution of signals for these 57 subjects is summarized in **Table 5**.

**Table 5. Distribution of Percentage of Cells with CEP 12 Signals in 57 Normal Peripheral Blood Specimens**

	Percent of Cells With				
	0 signal	1 signal	2 signals	3 signals	≥4 signals
Mean	0.34	3.36	96.06	0.20	0.04
SD	0.95	5.45	6.00	0.29	0.14

In cytogenetically normal peripheral blood specimens, the percentage of bi-signal and tri-signal cells are 2 critical categories for assessing expected values. The average percentages of bi-signal and tri-signal nuclei in the initial study were 97.39% (SD = 1.29%) and 0.36% (SD = 0.26%), respectively. Similarly, in the confirmatory study, the average percentages of bi-signal and tri-signal nuclei for the 57 normal specimens were 96.06% (SD = 6.00%) and 0.20% (SD = 0.29%), respectively. Thus, when the recommended enumeration guide is followed and practiced, the percent of bi-signal cells in a normal peripheral blood specimen should be between 84.3% and 100% (95% CI) and the percent of tri-signal cells should be between 0% and 0.87% (95% CI).

#### Values Among Peripheral Blood Specimens Positive for Trisomy 12

A study was performed to assess the distribution of interphase FISH signals in 39 (35 cultured and 4 uncultured) peripheral blood specimens previously determined to be positive for trisomy 12 by standard cytogenetic analysis. The distribution of signals for these 39 subjects is summarized in Table 6.

**Table 6.** Distribution of Percentage of Cells with CEP 12 Signals in 39 Peripheral Blood Specimens with Trisomy 12

	Percent of Cells With				
	0 signal	1 signal	2 signals	3 signals	≥4 signals
Mean	0.40	3.69	48.98	46.62	0.37
SD	1.21	5.80	14.27	17.99	0.60

In cytogenetically abnormal peripheral blood specimens, the critical category is the percent of cells with 3 signals (% tri-signal nuclei). Based on FISH analysis of the 39 specimens, the mean (±SD) % tri-signal nuclei was 46.62 (±17.99) and the median was 48.5%. The % tri-signal nuclei ranged from 7.2 to 79.2%.

#### Determination of the Cutoff

The other critical category in normal peripheral blood specimens is the percent tri-signal nuclei. The percent tri-signal nuclei in normal specimens was used for the determination of a cutoff point for trisomy 12. The percent of tri-signal cells was calculated for each of the 57 subjects in the pivotal study and the data distribution was assessed for normal distribution using the Shapiro-Wilk W Test.<sup>11</sup> The normal assumption was not satisfied and the data were log-transformed, and the mean percent of tri-signal cells and the standard deviation was calculated. The 95% 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 12

Statistics	Percent Tri-signal Nuclei	
	Log-transformed Values	Anti-log Arithmetic Value
Mean	-2.5998	—
Standard Deviation	1.5452	—
Upper 95% Confidence Interval	0.43	1.54
Mean + 1.96 SD		

The value for the cutoff point, 1.54%, calculated in Table 7 was rounded to 2.0%. Normal (negative) values are defined as ≤2.0 tri-signal cells, while >2.0% tri-signal cells are defined as positive for the presence of trisomy 12.

Prior to using the CEP 12 kit, the laboratory should verify its cutoff by analyzing and enumerating a minimum of 10 peripheral blood specimens according to the instructions in the enumeration section of the package insert. The percent tri-signal nuclei in these normal specimens should all be below the cutoff point of 2.0%. 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 pivotal study, the average percent of cells with no hybridization signal was 0.43% (SD = 1.48%) on 402 peripheral blood specimens. Thus, <2% cells with no signal is a realistic standard of acceptance.

#### Analytical Sensitivity

The analytical sensitivity of the CEP 12 probe was tested in the reproducibility study described below. In that study, the 0% specimen was estimated with a mean of 1.72% (SD = 1.09%) tri-signal nuclei and the 5% specimen, 4.87% (SD = 0.99%). There was slight overlap between the 0% and 5% specimens; the upper 95% confidence limit for the 0% specimen was 3.86% and the lower 95% confidence limit for the 5% specimen was 2.93%. Thus, the limit of detection for CEP 12 is estimated to be 4.0%.

#### Analytical Specificity

Locus specificity studies were performed with metaphase spreads according to standard Abbott Molecular Quality Control (QC) protocols. A total of 56 metaphase spreads were examined sequentially by G-banding to identify chromosome 12, followed by FISH. No cross-hybridization to other chromosome loci was observed in any of the 56 cells examined; hybridization was limited to the centromere region of chromosome 12.

#### Reproducibility

To assess the reproducibility of the CEP 12 assay, CEP 12 analyses for the percent of tri-signal cells were assessed for intersite, interlot, interday, and interobserver reproducibility. Four mixtures of hematologically derived human cells with known percent of trisomy 12 (approximately 0%, 5%, 10%, and 13%) were evaluated for the percent of tri-signal cells according to the instructions for signal enumeration in the package insert.

From the intra-assay study, the mean value of observed percentage of tri-signal nuclei for each specimen, the SD representing the intra-assay variability, and percent CV representing the precision are shown in Table 8. From the interassay study, the mean, SD, and percent CV of the observed percentage of tri-signal nuclei by study site, probe lot, assay day, and observer are shown in Tables 9 through 12. There were statistically significant observer-to-observer and site-to-site variations, reflecting the subjectivity of the visual enumeration.

**Table 8.** Precision of the Observed % Tri-signal Nuclei

Level of Trisomy 12	n	Mean	Standard Deviation	Coefficient of Variation
0%	22	1.72%	1.09%	63.1%
5%	23	4.87%	0.99%	20.4%
10%	23	9.19%	1.78%	19.4%
13%	24	12.07%	1.61%	13.3%

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

Level of Trisomy 12	Statistics	Site #1	Site #2	Site #3
0%	Mean	1.15	0.88	1.68
	SD	0.70	0.40	1.30
	CV(%)	60.78	45.46	77.44
	n	8	8	8
5%	Mean	4.10	3.89	4.77
	SD	1.15	1.31	0.74
	CV(%)	28.08	33.64	15.42
	n	8	8	8
10%	Mean	8.95	8.56	8.74
	SD	1.91	1.42	1.35
	CV(%)	21.36	16.62	15.49
	n	8	8	8
13%	Mean	12.36	11.29	12.88
	SD	2.93	2.56	1.92
	CV(%)	23.67	22.71	14.94
	n	8	8	8

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

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

Level of Trisomy 12	Statistics	Lot #1	Lot #2	Lot #3	Lot #4
0%	Mean	0.99	1.13	1.56	1.26
	SD	0.34	0.67	1.51	0.87
	CV(%)	34.38	58.88	96.72	68.87
	n	6	6	6	6
5%	Mean	4.30	4.99	4.46	3.28
	SD	0.42	0.85	1.15	1.28
	CV(%)	9.79	17.11	25.80	39.02
	n	6	6	6	6
10%	Mean	9.37	8.47	8.07	9.08
	SD	1.01	2.04	1.48	1.42
	CV(%)	10.78	24.08	18.36	15.62
	n	6	6	6	6
13%	Mean	11.04	12.86	12.57	12.23
	SD	2.70	3.48	2.04	1.60
	CV(%)	24.47	27.08	16.22	13.05
	n	6	6	6	6

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

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

Level of Trisomy 12	Statistics	Assay Day #1	Assay Day #2	Assay Day #3	Assay Day #4
0%	Mean	1.92	1.22	0.73	1.06
	SD	1.49	0.38	0.33	0.63
	CV(%)	77.67	31.29	44.65	59.54
	n	6	6	6	6
5%	Mean	4.02	4.49	4.50	4.02
	SD	1.45	1.02	0.81	1.28
	CV(%)	36.08	22.67	18.05	31.75
	n	6	6	6	6
10%	Mean	9.47	8.13	9.02	8.37
	SD	1.58	1.48	1.47	1.56
	CV(%)	16.69	18.21	16.27	18.67
	n	6	6	6	6
13%	Mean	10.71	11.86	13.56	12.56
	SD	1.35	3.02	1.59	3.14
	CV(%)	12.55	25.46	11.72	24.98
	n	6	6	6	6

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

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

Level of Trisomy 12	Statistics	Observer #1	Observer #2
0%	Mean	1.14	1.33
	SD	0.58	1.17
	CV(%)	50.42	88.21
	n	12	12
5%	Mean	4.60	3.91
	SD	1.18	0.96
	CV(%)	25.74	24.61
	n	12	12
10%	Mean	8.76	8.74
	SD	1.51	1.60
	CV(%)	17.25	18.27
	n	12	12
13%	Mean	12.31	12.04
	SD	2.45	2.63
	CV(%)	19.88	21.82
	n	12	12

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

## Methods Comparison; Clinical Specimens

A multicenter, blinded, comparative study was conducted to further define the performance of the CEP 12 SpectrumOrange DNA probe kit relative to standard cytogenetic analysis. Peripheral blood specimens were obtained from a total of 402 patients with B-cell chronic lymphocytic leukemia (B-CLL) for standard cytogenetic and FISH analysis. Specimens were evaluated at 3 sites; 97 specimens were analyzed at site 1, 205 at site 2, and 100 at site 3. All sites used cultured specimens for standard cytogenetic and FISH analyses except site 1; it used direct preparations for FISH only, but the same patient specimen was used for both methods. Each site followed its own in-house protocol for standard cytogenetic analysis; FISH analyses were performed according to the instructions in the CEP 12 SpectrumOrange DNA probe kit package insert\*. At site 3, 11 of the 100 specimens, on initial evaluation, showed >2% of cells with no signal. The enumeration was repeated on these specimens; the repeat enumeration results were acceptable, and were used in the analysis.

\*Site 2 used a slight modification to the recommended time and temperature for the FISH hybridization step.

A total of 177 specimens had a sufficient number of metaphase cells ( $\geq 20$ , or at least 2 metaphases with trisomy 12) for standard cytogenetic analysis. In addition, 157 specimens from 1 site had insufficient metaphases for complete cytogenetic analysis but were evaluated by FISH (see Table 14).

Of those specimens with sufficient metaphases for analysis, 41 were classified as positive for trisomy 12; 32 negative; and 4 ambiguous (1 trisomy 12 cell per 30 metaphases), by standard cytogenetic analysis. By interphase FISH analysis, 53 specimens were classified as positive for trisomy 12; 119 were negative; and 5 were uninformative (with less than 500 evaluable interphase nuclei).

When results between interphase FISH and standard cytogenetics were compared using only specimens with sufficient metaphases for analysis, the CEP 12 DNA probe kit showed a relative sensitivity of 100% (95% CI 91.2 to 100%), and a relative specificity of 91.47% (95% CI 86.66 to 96.22%). FISH interphase analysis designated 54 specimens as positive; 13 more than were positive by standard cytogenetic analysis. The results are shown in Table 13.

**Table 13. Classification of Trisomy 12 by CEP 12 versus Standard Cytogenetic Analysis, Specimens with Sufficient Metaphases Only**

CEP 12 Interphase Analysis <sup>a</sup>	Standard Cytogenetic Analysis			
	Positive	Negative	Ambiguous	Total
Positive	39 (100%)	11	4	54 (31.40%)
Negative	0	118 (91.47%)	0	118
Uninformative <sup>b</sup>	2	3	0	5
<b>TOTAL</b>	<b>41</b>	<b>132</b>	<b>4</b>	<b>177</b>

<sup>a</sup> Cutoff point is 2.0%.

<sup>b</sup> Results from specimens with insufficient interphase cells for FISH were not included in the analysis of relative sensitivity and relative specificity.

One study site (from the United Kingdom) evaluated a significant number of specimens (157) with less than the minimum number (20) of metaphases required by the International System for Human Cytogenetic Nomenclature (ISCN) standards for standard cytogenetic analysis. At this site, a minimum of 10 metaphase cells were examined and specimens were designated as negative or ambiguous if 0 or 1 cell, respectively, showed trisomy 12. If >2 cells were positive for trisomy 12, the specimen was reported as positive, and these results were included in Table 13. FISH analysis designated 25 of these 157 specimens as positive for trisomy 12; they were reported as negative or ambiguous at this site by standard cytogenetic analysis.



**Table 14.** Classification of Trisomy 12 by CEP 12 versus Standard Cytogenetic Analysis, Specimens with Insufficient Metaphases Only

CEP 12 Interphase Analysis <sup>a</sup>	Standard Cytogenetic Analysis			
	Positive	Negative	Ambiguous	Total
Positive	—	19	6	25 (16%)
Negative	0	130 (87%)	2	132
<b>TOTAL</b>	—	149	8	157

<sup>a</sup> Cutoff point is 2.0%.

Although the true status of specimens designated as trisomy 12 by FISH and negative or ambiguous by standard cytogenetic analysis has not been established, these discrepancies may, in part, be a reflection of the difference in the reported analytic sensitivity between the 2 methods.

#### Distribution of Tri-signalized Nuclei by Site

The distribution of the percent tri-signalized nuclei among the 3 sites, for the 94 positive cases by FISH interphase analysis, showed statistically significant site-to-site variation. The mean ( $\pm$ SD) % tri-signalized nuclei was 42.6% ( $\pm$ 24.0%), 44.9% ( $\pm$ 17.7%), and 28.6% ( $\pm$ 14.3%), for sites 1, 2, and 3, respectively.

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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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\*Vysis LSI, CEP, and WCP direct-label fluorescence probes are covered by US Patent 5,491,224.

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February 2013  
30-608311/R2