**MYC Break Apart DNA-FISH Probe**

**Two Color, Break Apart Probe**

**Instructions for Use**

**Intended use**

The MYC Break Apart DNA-FISH Probe is designed to detect the translocation between the MYC gene located at 8q24 and one of 11 known translocation partners: loci using fluorescence in situ hybridization (FISH). This most common translocation, t(8;14)(q24;q32), is found in 75-85% of Burkitt lymphoma (BL) cases and is the cytogenetic hallmark of BL. Translocation of MYC is often detected as a secondary genomic abnormality at low frequencies in high-grade B-cell lymphomas, such as diffuse large B-cell lymphoma (DLBCL) (5-16%) and chronic lymphocytic leukemia (CLL) (0.1-2%). In DLBCL, the presence of a MYC translocation is associated with an aggressive disease with a poor prognosis and an unfavorable outcome. MYC translocation has also been observed in 4-6% of acute lymphoblastic leukemia (ALL). [1,2]

**Storage**

Storage of DNA-FISH Probe: Store at -20°C protected from light until the expiry date as indicated on the label.

Storage of Slides: Store hybridized slides at -20°C protected from direct light.

**Note:** The storage conditions apply to both opened and unopened products; vials stored under other conditions may not perform optimally and will affect the assay result. The number of freeze/thaw cycles should not exceed the recommended number of tests per vial. Store in original container.

**Handling**

- Handle all reagents as capable of transmitting infectious agents and dispose of according to current national law.
- Handle all reagents and slides containing fluorophores in reduced light to prevent photobleaching.

**Reagent provided**

Ready-to-use DNA-FISH Probe: 100 μL per vial (10 tests); one test is defined as sufficient for a 22 x 22 mm area.

The DNA-FISH Probe is premixed in hybridization buffer (formamide, dextran sulphate, and SSC) and contains fluorophore-labeled probes for the 5’ MYC locus (red) and 3’ MYC locus (green).

**Equipment**

<table>
<thead>
<tr>
<th>Coplin jars</th>
<th>Microcentrifuge</th>
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</thead>
<tbody>
<tr>
<td>Coverslip (22x22 mm)</td>
<td>Microfuge tube (0.5 mL)</td>
</tr>
<tr>
<td>2x5x25 mm</td>
<td>Micropipette (1-200 μL)</td>
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<tr>
<td>Epifluorescent microscope</td>
<td>pH meter</td>
</tr>
<tr>
<td>with appropriate filters</td>
<td>Rubber cement</td>
</tr>
<tr>
<td>Forceps</td>
<td>Slide tray</td>
</tr>
<tr>
<td>Fume hood</td>
<td>Slide warmer</td>
</tr>
<tr>
<td>Glove</td>
<td>Thermometer, calibrated</td>
</tr>
<tr>
<td>Humidified chamber</td>
<td>Hot plate</td>
</tr>
<tr>
<td>Immersion oil</td>
<td>Water bath</td>
</tr>
<tr>
<td>Incubator</td>
<td>Mercury lamp (100 watt)</td>
</tr>
</tbody>
</table>

**Reagents**

- 100% Ethanol
- 10X PBS
- 1N HCl
- 1M MgCl₂
- 1M NaOH
- 20X SSC
- 1% Formaldehyde
- 10% Formalin
- 100X MgCl₂
- 1N HCl
- 1M MgCl₂
- 1M NaOH
- 1% Formaldehyde
- 10% Formalin
- DAPI/Antifade
- Distilled Water
- Pepsin
- Tween 20

**Standard FISH Procedure**

**Note:** Products ready-to-use. Do not reconstitute or dilute with hybridization buffer. For professional use only.

- Only a technician familiar with cytogenetic methods and trained in the FISH technique can perform the assay. All equipment should be calibrated prior to performance of the assay.
- The intended tissue is peripheral blood and bone marrow. The slides should be prepared according to the guidelines for standard cytogenetic methods of the laboratory performing the assay.

**Slide Preparation**

All freshly prepared slides should be aged for 1.5 hours at 45-50°C before hybridization. If not hybridizing same day as prepared, store at +4°C or -20°C for long-term storage. Slides with visible cytoplasm may require pretreatment with proteolytic enzyme (see optional pretreatment with pepsin).

**Optional: Slide Pretreatment with Pepsin**

1. Pre-warm 50 mL of 0.01N HCl at 37°C.
2. Add 25 μL of 0.4% pepsin stock to the pre-warmed 50 mL of 0.01N HCl and incubate the slide for 5-10 min at 37°C in pepsin.

**Procedure Note:** Some specimens may require longer digestion time in pepsin or a higher concentration of pepsin.

3. Wash the slide twice for 5 min in 1X PBS at RT.
4. Incubate the slide for 5 min in 1% formaldehyde at RT.
5. Wash the slide twice for 5 min in 1X PBS at RT.
6. Dehydrate the slide in 70%, 85%, and 100% ethanol at RT for 1 min each.
7. Air dry the slide.

**Procedure Note:** Check the morphology of the sample with a phase contrast microscope before hybridization. Do not hybridize if the nuclear morphology is compromised.

**DNA-FISH Probe Denaturation / Hybridization**

1. Vortex the DNA-FISH Probe briefly and spin the tube in a microcentrifuge.
2. Add 10 μL of the DNA-FISH Probe to the target area and cover with a coverslip (22x22 mm).

**Procedure Note:** Care should be taken to avoid air bubbles. Smaller or larger coverslips may be used with proportional change in DNA-FISH Probe volume.

3. Seal the edges of the coverslip thoroughly with rubber cement.
4. Co-denature the slide and the DNA-FISH Probe for 3 min at 80°C on a temperature controlled hot plate or an automated hybridization device.
5. Incubate for 12-18 hours in a humidified environment at 37°C protected from direct light.

(Continued on the next page)
This product has been optimized for use on slides prepared from peripheral blood and bone marrow specimens according to routine cytogenetic methods. The manufacturer ensures that this product meets the analytical performance characteristics (sensitivity, specificity, reproducibility, and reportable range) established on intended tissues.

Each new lot of DNA-FISH Probe should be tested for locus specificity on a normal peripheral blood specimen, and on the intended tissue to verify proper reagent performance. It is the responsibility of the laboratory to establish the reportable ranges using positive and negative control specimens of the intended tissue.

Use of filters with spectral characteristics other than specified may adversely affect the strength of the signal. For example, the red fluorophore is visible through an orange filter, but the signals appear dim.

Metaphase FISH is recommended to characterize variant and atypical abnormal signal pattern.

The FISH assay is considered an adjunct to classical cytogenetics (karyotyping). The results of these assays must be interpreted in the full context of the patient's clinical history. A medical decision cannot be made based on the result of the FISH assay alone.

Signal visualization and interpretation

The signal should be visualized with an epi-fluorescence microscope equipped with the appropriate filters.

**Procedure Note:** The signals can be at different focal plane so it is important to focus up and down on the specimen to ensure that all the signals are counted.

In normal diploid metaphase and interphase nucleus, two fusion signals (red/green or yellow) would be observed corresponding to the normal homologous chromosome 8.

In cells with chromosomal rearrangements involving the MYC gene, the most commonly observed pattern is one fusion signal (red/green or yellow), which represents the normal chromosome 8, and one red and one green signal, which represents the derivative chromosomes.

**Symbol Glossary**

- **LOT** Batch Code
- **EC** CE marking of conformity
- **H** Upper limit of temperature
- **C** Caution, consult accompanying documents
- **V** In Vitro Diagnostic medical device
- **M** Manufacturer
- **Use By**
- **Keep away from sunlight**
- **Contains sufficient for 10 tests**
- **In Vitro**

**Filter Requirements**

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>Excitation max</th>
<th>Emission max</th>
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<tbody>
<tr>
<td>Green</td>
<td>496 nm</td>
<td>520 nm</td>
</tr>
<tr>
<td>Red</td>
<td>580 nm</td>
<td>603 nm</td>
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<tr>
<td>DAPI</td>
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**Recommendations and limitations**

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**References**