

# A20/PRDM1/SHGC-79576 DNA-FISH Probe

## Three Color, Enumeration Probe

REF 19-001

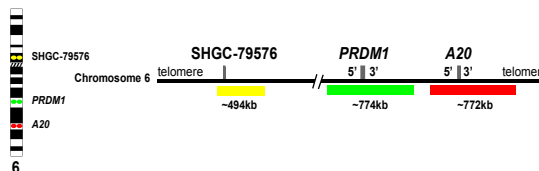


### Instructions for Use



#### Intended use

The A20 (also called TNFAIP3)/PRDM1/SHGC-79576 DNA-FISH Probe is designed to detect deletion of the A20 gene located on 6q23 and the PRDM1 gene located on 6q21 relative to the control locus SHGC-79576 on 6p12, using fluorescence *in situ* hybridization (FISH). Deletion of 6q is observed in all types of B-cell malignancies, where two commonly deleted regions map to the A20 and PRDM1 gene region.<sup>[1,2]</sup> Loss of the A20 gene has been observed in ~20% of non-Hodgkin lymphoma (NHL) cases, ~20% of mucosa-associated lymphoid tissue (MALT) lymphoma cases<sup>[3]</sup> and in nonsplenic marginal zone lymphomas (MZLs) cases.<sup>[4]</sup> The loss of the A20 gene has been observed most frequently in mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL) cases with a frequency rate of 31% and 38%, respectively.<sup>[5]</sup> In DLBCL, inactivation of is seen more frequently in the activated B-cell (ABC) subtype of (50%) versus the germinal center B-cell (GCB) subtype (22%).<sup>[3,5,6]</sup> DLBCL cases exhibit a variety of complex 6q deletions, which encompass either A20 or PRDM1 alone or together as part of a larger deletion.<sup>[2]</sup> Additionally, the deletion of the PRDM1 gene has been observed in 53% of primary central nervous system lymphomas (PCNSLs).<sup>[7,8]</sup>



#### Schematic of the A20/PRDM1/SHGC-79576 DNA-FISH Probe:

Horizontal red, green, and gold bars indicate the regions covered by the probes (approximate to scale, GRCh37/Hg19/2009). The directly labeled A20 (red) and PRDM1 (green) span the respective genes and SHGC-79576 (gold) serves as a control.

#### 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.

#### Warnings and Precautions

**Please read instructions before proceeding.**

- » Improper transportation or storage can destroy or impair the performance of the product.
- » If any of the following occurs: receipt of compromised package or vial, device failure (when used according to instructions for use), or injury of user, please contact the manufacturer.
- » Any compromised vial should be discarded according to current national law and assays should not be performed from such reagent.
- » Handle all reagents with care and wear appropriate personal protective equipment.
- » Formamide, Saline-Sodium Citrate (SSC), and Sodium Dodecyl Sulfate (SDS) may have teratogenic and mutagenic effects: avoid inhalation, ingestion or contact with skin.
- » DAPI is an irritant. See Material Safety Data Sheet (MSDS) for safety information.

#### 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 A20 locus (red), PRDM1 locus (green), and SHGC-79576 locus (yellow).

#### Reagent, Material, and Equipment required but not provided

Equipment	Reagents
Coplin jars	100% Ethanol
Coverslip (22x22 mm & 25x25 mm)	10X PBS
Epi-fluorescent microscope with appropriate filters	1N HCl
Forceps	1M MgCl <sub>2</sub>
Fume hood	1M NaOH
Gloves	20X SSC
Humidified chamber	10% Formalin
Immersion oil	DAPI/Antifade
Incubator	Distilled Water
Mercury lamp (100 watt)	Pepsin
	Tween 20

#### Reagent preparation

**Note:** Use distilled water for the preparation of all stock and working solutions.

**Ethanol Series (70%, 85%, and 100%):** Prepare v/v dilutions of 100% ethanol with distilled water (dH<sub>2</sub>O). Store at RT.

**0.01N HCl (Hydrochloric acid):** Add 0.5 mL of 1N HCl to 49.5 mL (dH<sub>2</sub>O). Store at RT. Pre-warm the solution to 37°C in a waterbath.

**0.4% (4 mg/mL) Pepsin Stock Solution:** Dissolve 100 mg of pepsin in 25 mL 0.2N HCl. Store 500 µL aliquots at -20°C.

**1% Formaldehyde:** Add 12.5 mL of 10% formalin (4% formaldehyde) to 37 mL of 1X PBS. Add 500 µL of 100X MgCl<sub>2</sub>. Store up to 1 week at 4°C.

**0.5X SSC (Saline-Sodium Citrate)/0.1% Tween 20:** Add 25 mL of 20X SSC and 1 mL of Tween 20 to 974 mL dH<sub>2</sub>O. Mix well by swirling. Store at RT.

**1X PBS (Phosphate Buffer Saline):** Mix 100 mL of 10X PBS to 900 mL dH<sub>2</sub>O. Adjust pH to 7.0. Store at RT.

**2X SSC:** Mix 100 mL of 20X SSC and 900 mL of dH<sub>2</sub>O. Adjust pH to 7.0. Store at room temperature (RT).

**2X SSC/0.1% Tween 20:** Add 100 mL of 20X SSC and 1 mL of Tween 20 to 899 mL dH<sub>2</sub>O. Mix well by swirling. Store at RT.

**100X MgCl<sub>2</sub> (Magnesium Chloride) in 1X PBS:** Add 50 µL of 1M MgCl<sub>2</sub> to 450 µL of 1X PBS.

#### Standard FISH Procedure

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

- » Only a technologist familiar with cytogenetic methods and trained for 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 room temperature (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.

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## Standard FISH Procedure (con't)

### DNA-FISH Probe Denaturation / Hybridization

1. Vortex the DNA-FISH Probe briefly and spin the tube in a microcentrifuge.
2. Apply 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.

### Microscope accessories

#### » Objectives

A 10X objective is suitable for scanning the target area. Higher magnification is required for signal analysis and should be performed with a 63X or a 100X oil immersion objective.

#### » Immersion oil

The immersion oil should be suitable for fluorescence microscopy.

#### » Lamp

A 100 watt mercury lamp with a maximum life of 200 hours is recommended. Replace the lamp before it exceeds 200 hours.

### 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 red, two green, and two gold signals would be observed corresponding to the two normal homologous chromosome 6.
- » Upon interstitial deletion of 6q23, in which the *A20* gene is deleted and the 6q21 (*PRDM1*) and 6p12 (SHGC-79576) loci remains, one red, two green, and two gold signals would be observed.
- » However, upon interstitial deletion of 6q21, in which the *PRDM1* gene is deleted and the 6q23 (*A20*) and 6p12 (SHGC-79576) loci remains, two red, one green, and two gold signals would be observed.
- » Upon deletion of an entire chromosome 6, a single red, green, and gold signal would be observed, which corresponds to the remaining chromosome 6.

### Post Hybridization Washing

**Procedure Note:** Do not allow the slide to dry before washes are complete.

1. Pre-warm the 2X SSC/0.1% Tween 20 and 0.5X SSC/0.1% Tween 20 solutions at 45°C.
2. Remove the rubber cement from the slide with forceps.
3. Remove the cover-slip by briefly soaking in 2X SSC at RT.
4. Wash the slide in two changes of 2X SSC/0.1% Tween 20 for 5 min at 45°C.
5. Wash the slide in two changes of 0.5X SSC/0.1% Tween 20 for 5 min at 45°C.
6. Briefly rinse the slide in dH<sub>2</sub>O.
7. Air dry the slide out of direct light.
8. Apply 20 µL of DAPI/Antifade solution to the hybridized area and cover with a coverslip (25x25 mm).












#### » Filter Requirements

Fluorophore	Excitation <sub>max</sub>	Emission <sub>max</sub>
Green	496 nm	520 nm
Red	593 nm	612 nm
Yellow	525 nm	551 nm
DAPI	360 nm	460 nm

### Recommendations and limitations

- » 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.

### Symbol Glossary

 LOT	Batch Code		Contains sufficient for 10 tests
	Biological risks		In Vitro Diagnostic medical device
	Catalogue number		Keep away from sunlight
	Caution, consult accompanying documents		Manufacturer
	CE marking of conformity		Upper limit of temperature
			Use By



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DNA-FISH Probe manufactured in EU by Cancer Genetics Italia S.r.l.

### References

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