

Recommended FISH protocol for Cytocell Aquarius® AML and MDS FISH Probe Kits only

Reference the Package Insert (Instructions for Use (IFU)) for warnings, precautions, storage, and handling.

Study the Package Insert carefully before using this quick reference guide.

This can be found in the product packaging and by using the Resources section of the Cytocell website (www.cytocell-us.com/resources).

This quick reference guide does not replace the content from the Package Insert.

Probe Name	Cat. No.*	Package Insert
AML1/ETO (RUNX1/RUNX1T1) Translocation, Dual Fusion	USA-LPH 026	DS059/USA
CBFβ (CBFB)/MYH11 Translocation, Dual Fusion	USA-LPH 022	DS060/USA
Del(5q) Deletion	USA-LPH 024	DS061/USA
Del(7q) Deletion	USA-LPH 025	DS062/USA
Del(20q) Deletion	USA-LPH 020	DS076/USA
EVI1 (MECOM) Breakapart	USA-LPH 036	DS106/USA
MLL (KMT2A) Breakapart	USA-LPH 013	DS083/USA
P53 (TP53) Deletion	USA-LPH 017	DS086/USA

*Kit includes FISH probe and DAPI

The Cytocell Aquarius AML/MDS range of FISH probe test kits are fluorescence *in situ* hybridization (FISH) tests used to detect common chromosomal rearrangements in fixed bone marrow specimens from patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). The tests are indicated for the characterization of patient specimens consistent with World Health Organization guidelines for Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th Edition) and in conjunction with other clinicopathological criteria. The assay results are to be interpreted by a qualified pathologist or cytogeneticist. The tests are not intended for use as a stand-alone diagnostic, disease screening, or as a companion diagnostic.







Refer to individual test kit Package Insert for the specific intended use and limitations.

For *In Vitro* Diagnostic Use. Rx only.

Materials Provided

The FISH probes are provided in a 100µl (10 test) per vial format. They are supplied ready-to-use, premixed with hybridization solution (formamide; dextran sulfate; saline-sodium citrate (SSC)). In addition a 150µl vial of ready-to-use DAPI counterstain with antifade (0.125µg/ml DAPI (4,6-diamidino-2-phenylindole)) is provided. See Package Insert for additional details.

Protocol Steps

Step 1		Sample and slide preparation <ul style="list-style-type: none">• The FISH probes for AML/MDS are designed for use on bone marrow cells fixed in Carnoy's solution (3:1 methanol/ acetic acid) that are prepared according to the laboratory or institution guidelines.• Spot the cell sample onto a glass microscope slide. Allow to dry.• Immerse the slide in 2x Saline Sodium Citrate (SSC) for 2 minutes at room temperature (RT) without agitation.• Dehydrate in an ethanol series (70%, 85% and 100%), each for 2 minutes at RT.• Allow to dry.
Step 2		Pre-denaturation <ul style="list-style-type: none">• Remove the probe from the freezer and allow it to warm to RT. Briefly centrifuge tubes before use.• Ensure that the probe solution is sufficiently mixed with a pipette or a vortex mixer.• Remove 10µl of probe per test, and transfer it to a microcentrifuge tube. Quickly return the remaining probe to -20°C.• Place the probe and the sample slide to prewarm on a 37°C (+/- 1°C) hotplate for 5 minutes.• Spot 10µl of probe mixture onto the cell sample and carefully apply a 24x24mm coverslip. Seal with rubber solution glue and allow the glue to dry completely.
Step 3		Denaturation <ul style="list-style-type: none">• Denature the sample and probe simultaneously by heating the slide on a hotplate at 75°C (+/- 1°C) for 2 minutes.
Step 4		Hybridization <ul style="list-style-type: none">• Place the slide in a humid, lightproof container at 37°C (+/- 1°C) overnight.
Step 5		Post-hybridization washes <ul style="list-style-type: none">• Remove the DAPI from the freezer and allow it to warm to RT.• Remove the coverslip and all traces of glue carefully.• Immerse the slide in 0.4x Saline Sodium Citrate (SSC) (pH 7.0) at 72°C (+/- 1°C) for 2 minutes without agitation.• Drain the slide and immerse it in 2xSSC + 0.05% Tween-20 at RT (pH 7.0) for 30 seconds without agitation.• Drain the slide and apply 10µl of DAPI antifade onto each sample.• Cover with a 24x24mm coverslip, remove any bubbles.• Edge the slide with clear nail varnish to seal.• Allow the color to develop in the dark for 10 minutes.
Step 6		Analyze <ul style="list-style-type: none">• View with a fluorescence microscope.• For optimal visualization of the probes, a 100-Watt mercury lamp (or equivalent) is recommended with plan apochromat objectives 63x or 100x.• Filters designed specifically for detection of DAPI, FITC, Texas Red®, and Aqua or DEAC fluorophores individually or in combination (e.g. dual or triple filters) are optimal for best results.• The final hybridized slides are analyzable for up to 1 month when stored in darkness and at 2-8°C.

For more information and links to technical support, visit www.cytocell-us.com/AML

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