A Sysmex Group Company

CytoCell

Instructions For Use

REF: RU-LPH 108-S / RU-LPH 108

IGH/MAF *Plus* v2 Translocation, Dual Fusion Probe

Research Use Only

PROFESSIONAL USE ONLY

Further information available at www.ogt.com

Principles of the Test

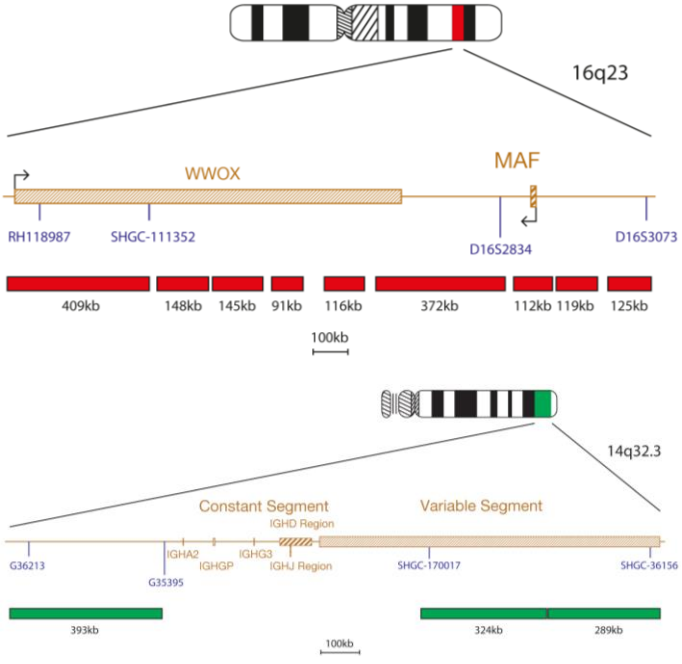
Fluorescence *in situ* hybridisation (FISH) is a technique that allows DNA sequences to be detected on metaphase chromosomes or in interphase nuclei from fixed cytogenetic samples. The technique uses DNA probes that hybridise to entire chromosomes or single unique sequences, and serves as a powerful adjunct to G-banded cytogenetic analysis. This technique can now be applied as an essential investigative tool within prenatal, haematological and solid tumour chromosomal analysis. Target DNA, after fixation and denaturation, is available for annealing to a similarly denatured, fluorescently labelled DNA probe, which has a complementary sequence. Following hybridisation, unbound and non-specifically bound DNA probe is removed and the DNA is counterstained for visualisation. Fluorescence microscopy then allows the visualisation of the hybridised probe on the target material.

Intended Use

This product is intended to be used for research use only and is not for use in diagnostic procedures.

Probe Specification

MAF, 16q23, Red
IGH, 14q32.3, Green



The IGH/MAF *Plus* v2 Translocation, Dual Fusion Probe consists of the IGH probe mix, labelled in green, proximal to the Constant segment and within the Variable segment of the IGH region and the MAF probe mix, labelled in red, that encompasses the MAF gene and flanking regions as well as the WWOX gene.

Materials Provided

Probe: 50µl per vial or 100µl per vial
The probes are provided premixed in hybridisation solution (formamide; dextran sulphate; saline-sodium citrate (SSC)) and are ready to use.

Counterstain: 150µl per vial
The counterstain is DAPI antifade (ES: 0.125µg/ml DAPI (4,6-diamidino-2-phenylindole)).

Warnings and Precautions

1. For research use only. Not for use in diagnostic procedures.
2. For professional use only.
3. Wear gloves when handling DNA probes and DAPI counterstain.
4. Probe mixtures contain formamide, which is a teratogen; do not breathe fumes or allow skin contact. Handle with care; wear gloves and a lab coat.
5. DAPI is a potential carcinogen. Handle with care; wear gloves and a lab coat.
6. Dispose of all hazardous materials according to your institution's guidelines for hazardous waste disposal.
7. Operators must be capable of distinguishing the colours red, blue and green.
8. The probe should not be diluted or mixed with other probes.

Storage and Handling

The kit should be stored between -25ºC to -15ºC in a freezer until the expiry date indicated on the kit label. The probe and counterstain vials must be stored in the dark.

The probe remains stable throughout the freeze-thaw cycles experienced during normal use (where one cycle constitutes the probe's removal from and replacement into the freezer) and is photostable for up to 48 hours after being exposed to continuous lighting conditions. All efforts must be made to limit exposure to light and temperature changes.

Equipment and Materials Necessary but not Supplied

Calibrated equipment must be used:

1. Hotplate (with a solid plate and accurate temperature control up to 80ºC)
2. Calibrated variable volume micropipettes and tips range 1µl - 200µl
3. Water bath with accurate temperature control at 37ºC and 72ºC
4. Microcentrifuge tubes (0.5ml)
5. Fluorescence microscope (Please see Fluorescence Microscope Recommendation section)
6. Phase contrast microscope
7. Clean plastic, ceramic or heat-resistant glass Coplin jars
8. Forceps
9. Calibrated pH meter (or pH indicator strips capable of measuring pH 6.5 – 8.0)
10. Humidified container
11. Fluorescence grade microscope lens immersion oil
12. Bench top centrifuge
13. Microscope slides
14. 24x24mm coverslips
15. Timer
16. 37ºC incubator
17. Rubber solution glue
18. Vortex mixer
19. Graduated cylinders
20. Magnetic stirrer
21. Calibrated thermometer

Optional Equipment not Supplied

1. Cytogenetic drying chamber

Reagents Needed but not Supplied

1. 20x saline-sodium citrate (SSC) Solution
2. 100% Ethanol
3. Tween-20
4. 1M Sodium hydroxide (NaOH)
5. 1M Hydrochloric acid (HCl)
6. Purified water

Fluorescence Microscope Recommendation

Use a 100-watt mercury lamp or equivalent and oil immersion plan apochromat objectives 60/63x or 100x for optimal visualisation. The fluorophores used in this probe set will excite and emit at the following wavelengths:

Fluorophore	$Excitation_{max}$ [nm]	$Emission_{max}$ [nm]
Green	495	521
Red	596	615

Ensure appropriate excitation and emission filters that cover the wavelengths listed above are fitted to the microscope. Use a triple bandpass DAPI/green spectrum/red spectrum filter or a dual bandpass green spectrum/red spectrum filter for optimal simultaneous visualisation of the green and red fluorophores.

Check the fluorescence microscope before use to ensure it is operating correctly. Use immersion oil that is suitable for fluorescence microscopy and formulated for low auto fluorescence. Avoid mixing DAPI antifade with microscope immersion oil as this will obscure signals. Follow manufacturers' recommendations in regards to the life of the lamp and the age of the filters.

Sample Preparation
The kit is designed for use on haematologically-derived cell suspensions fixed in Carnoy's solution (3:1 methanol/acetic acid) fixative, that are prepared according to the laboratory or institution guidelines. Prepare air dried samples on microscope slides according to standard cytogenetic procedures. The AGT *Cytogenetics Laboratory Manual* contains recommendations for specimen collection, culturing, harvesting and for slide making[1].

Solution Preparation
Ethanol Solutions
Dilute 100% ethanol with purified water using the following ratios and mix thoroughly:
- 70% Ethanol - 7 parts 100% ethanol to 3 parts purified water
- 85% Ethanol - 8.5 parts 100% ethanol to 1.5 parts purified water

Store the solutions for up to 6 months at room temperature in an airtight container.

2xSSC Solution
Dilute 1 part 20xSSC Solution with 9 parts purified water and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

0.4xSSC Solution
Dilute 1 part 20xSSC Solution with 49 parts purified water and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

2xSSC, 0.05% Tween-20 Solution
Dilute 1 part 20xSSC Solution with 9 parts purified water. Add 5µl of Tween-20 per 10ml and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

FISH Protocol
(Note: Ensure that exposure of the probe and counterstain to laboratory lights is limited at all times).

Slide Preparation
1. Spot the cell sample onto a glass microscope slide. Allow to dry. (**Optional, if using a cytogenetic drying chamber**: slides should be spotted using a cytogenetic drying chamber. The chamber should be operated at approximately 25°C and 50% humidity for optimal cell sample spotting. If a cytogenetic drying chamber is not available, use a fume hood as an alternative).
2. Immerse the slide in 2xSSC for 2 minutes at room temperature (RT) without agitation.
3. Dehydrate in an ethanol series (70%, 85% and 100%), each for 2 minutes at RT.
4. Allow to dry.

Pre-Denaturation
5. Remove the probe from the freezer and allow it to warm to RT. Briefly centrifuge tubes before use.
6. Ensure that the probe solution is uniformly mixed with a pipette.
7. Remove 10µl of probe per test, and transfer it to a microcentrifuge tube. Quickly return the remaining probe to the freezer.
8. Place the probe and the sample slide to prewarm on a 37ºC (+/- 1ºC) hotplate for 5 minutes.
9. Spot 10µl of probe mixture onto the cell sample and carefully apply a coverslip. Seal with rubber solution glue and allow the glue to dry completely.

Denaturation
10. Denature the sample and probe simultaneously by heating the slide on a hotplate at 75ºC (+/- 1ºC) for 2 minutes.

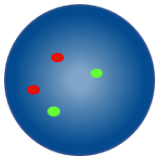
Hybridisation
11. Place the slide in a humid, lightproof container at 37ºC (+/- 1ºC) overnight.

Post-Hybridisation Washes
12. Remove the DAPI from the freezer and allow it to warm to RT.
13. Remove the coverslip and all traces of glue carefully.
14. Immerse the slide in 0.4xSSC (pH 7.0) at 72ºC (+/- 1ºC) for 2 minutes without agitation.
15. Drain the slide and immerse it in 2xSSC, 0.05% Tween-20 at RT (pH 7.0) for 30 seconds without agitation.
16. Drain the slide and apply 10µl of DAPI antifade onto each sample.
17. Cover with a coverslip, remove any bubbles and allow the colour to develop in the dark for 10 minutes.
18. View with a fluorescence microscope (see **Fluorescence Microscope Recommendation**).

Stability of Finished Slides
Finished slides remain analysable for up to 1 month if stored in the dark at/or below RT.

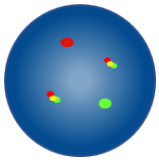
Procedural Recommendations
1. Baking or ageing of slides may reduce signal fluorescence
2. Hybridisation conditions may be adversely affected by the use of reagents other than those provided or recommended by Cytocell Ltd.
3. Use a calibrated thermometer for measuring temperatures of solutions, waterbaths and incubators as these temperatures are critical for optimum product performance.
4. The wash concentrations, pH and temperatures are important as low stringency can result in non-specific binding of the probe and too high stringency can result in a lack of signal.
5. Incomplete denaturation can result in lack of signal and over denaturation can also result in non-specific binding.
6. Over hybridisation can result in additional or unexpected signals.
7. Users should optimise the protocol for their own samples prior to using the test for diagnostic purposes.
8. Suboptimal conditions may result in non-specific binding that may be misinterpreted as a probe signal.

Expected Results
Expected Normal Signal Pattern



In a normal cell, two red and two green signals (2R, 2G) are expected.

Expected Abnormal Signal Pattern



In a cell with a t(14;16)(q32.3;q23) translocation, the expected signal pattern will be one red, one green and two fusion signals (1R, 1G, 2F).

Other signal patterns are possible in aneuploid/unbalanced specimens. Please note that in the presence of other IGH rearrangements apart from the IGH/MAF translocation the green IGH signal may appear split.

Known Cross-Reactivity
The green IGH probe may show cross-reactivity to 15q11.2 and 16p11.2.

Additional Information
For additional product information please contact the CytoCell Technical Support Department.
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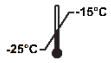
References
1. Arsham, MS., Barch, MJ. and Lawce HJ. (eds.) (2017) *The AGT Cytogenetics Laboratory Manual*. New Jersey: John Wiley & Sons Inc.

Guide to Symbols

REF	**en:** Catalogue number
LOT	**en**: Batch code
	**en:** Consult instructions for use
	**en:** Manufacturer
	**en:** Use-by date
	**en:** Temperature limit
	**en:** Keep away from sunlight
	**en:** Contains sufficient for <n> tests
CONT	**en:** Contents

Patents and Trademarks
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