A Sysmex Group Company

CytoCell

Instructions For Use
REF: RU-LPH 038-S / RU-LPH 038

BCR/ABL (ABL1) *Plus* Translocation, Dual Fusion Probe

Research Use Only

PROFESSIONAL USE ONLY

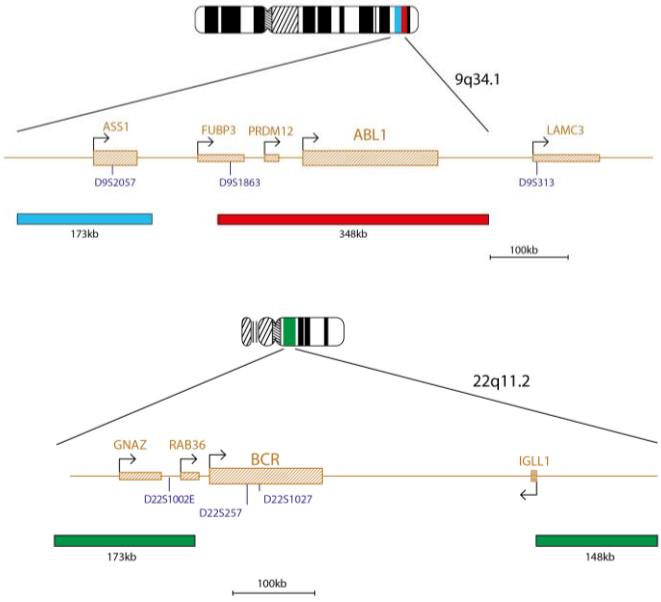
ENGLISH

Further information available at www.ogt.com

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 classic cytogenetics. Recent developments have meant that this valuable technique can now be applied as an essential tool in prenatal, haematological and pathological 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
ABL1, 9q34.11-q34.12, Red
BCR, 22q11.22-q11.23, Green
ASS1, 9q34.11-q34.12, Blue



The BCR/ABL1 probe mix contains a 173kb green probe centromeric to the BCR gene and contains the genes GNAZ and RAB36. A second green probe covers a 148kb region telomeric to the BCR gene and covers part of the IGLL1 gene. A red probe covers a 348kb region that spans the ABL1 gene. There is an additional blue probe that covers a 173kb region and spans the whole ASS1 gene.

Materials Provided
Probe: 50µl per vial or 100µl per vial
The probes are provided premixed in hybridisation solution (Formamide; Dextran Sulphate; 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. For professional use only.
2. Wear gloves when handling DNA probes and DAPI counterstain.
3. Probe mixtures contain formamide, which is a teratogen; do not breathe fumes or allow skin contact. Wear gloves, a lab coat, and handle in a fume hood. Upon disposal, flush with a large volume of water.
4. DAPI is a potential carcinogen. Handle with care; wear gloves and a lab coat. Upon disposal, flush with a large volume of water.
5. All hazardous materials should be disposed of according to your institution's guidelines for hazardous waste disposal.

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.

Protocol Recommendations

Equipment Necessary but not Supplied
1. Hotplate (with a solid plate and accurate temperature control up to 80ºC).
2. Variable volume micropipettes and tips range 1µl - 200µl.
3. Water bath with accurate temperature control at 72ºC.
4. Microcentrifuge tubes (0.5ml).
5. Fluorescence microscope (Please see Fluorescence Microscope Recommendation section).
6. Plastic or glass coplin jars.
7. Forceps.
8. Fluorescence grade microscope lens immersion oil.
9. Bench top centrifuge.
10. Microscope slides.
11. 24x24mm coverslips.
12. Timer.
13. 37ºC incubator.
14. Rubber solution glue.

Fluorescence Microscope Recommendation
For optimal visualisation of the probe we recommend a 100-watt mercury lamp and plan apochromat objectives x63 or x100. The Triple bandpass filter DAPI/FITC/Texas Red is optimal for viewing all fluorophores and DAPI simultaneously. The blue fluorophore has specificity to the Aqua and DEAC spectrum (single bandpass Aqua or DEAC filter is required).

Sample Preparation
Sample preparation should be performed according to the laboratory or institution guidelines.
Prepare air dried samples on microscope slides according to standard cytogenetic procedures.

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

Slide preparation
1. Spot the cell sample onto a glass microscope slide. Allow to dry.
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.
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 coverslip and all traces of glue carefully.
13. Immerse the slide in 0.4xSSC (pH 7.0) at 72ºC (+/- 1ºC) for 2 minutes without agitation.
14. Drain the slide and immerse it in 2xSSC, 0.05% Tween-20 at RT (pH 7.0) for 30 seconds without agitation.
15. Drain the slide and apply 10µl of DAPI antifade onto each sample.
16. Cover with a coverslip, remove any bubbles and allow the colour to develop in the dark for 10 minutes.
17. View with a fluorescence microscope.

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

Procedural Recommendations

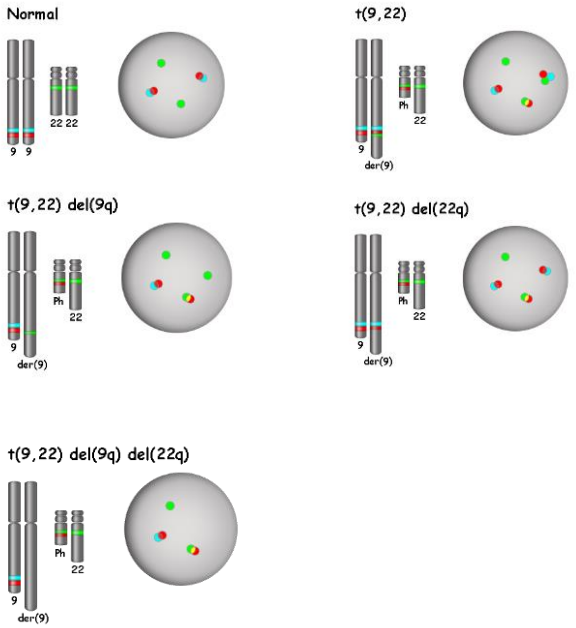
1. Baking or ageing of slides is not recommended as it may reduce signal fluorescence.
2. Hybridisation conditions may be adversely affected by the use of reagents other than those provided or recommended by Cytocell Ltd.
3. The use of a calibrated thermometer is strongly recommended for measuring temperatures 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.

Expected Results

In a normal cell these probes should appear as discrete red/blue and green spots, one for each homologue (resulting in a 2RB, 2G conformation). Depending on the rearrangement, cells can show one of the following signal patterns:

1. In a classical t(9;22)(q34;q11) case (without deletion) one red/green (yellow fusion) and one red/green/blue signal in addition to one red/blue and one green signal of the normal chromosomes 9 and 22 respectively should be observed (1RG, 1RGB, 1RB and 1G).
2. In a t(9;22)(q34;q11) cell with loss of proximal 9q one red/green (yellow fusion) and one green signal in addition to one red/blue and one green signal of the normal chromosomes 9 and 22 respectively should be observed (1RG, 2G and 1RB).
3. In a t(9;22)(q34;q11) cell with loss of distal 22q one red/green (yellow fusion) and one red/blue signal in addition to one red/blue and one green signal of the normal chromosomes 9 and 22 respectively should be observed (1RG, 2RB and 1G).
4. In a t(9;22)(q34;q11) cell with loss of proximal 9q and distal 22q one red/green (yellow fusion) signal in addition to one red/blue and one green signal of the normal chromosomes 9 and 22 respectively should be observed (1RG, 1RB and 1G).

The ASS1 probe in blue can differentiate random signal overlap from true BCR/ABL1 fusion in the interphase cells. The random signal overlap would result in the presence of the blue signal, while the true fusion would result in the absence of the blue signal.



Known Cross-Reactivity

The green BCR distal probe may show up to 2 cross-hybridisation signals on chromosome 7 at 7q11.2.

Additional Information

For additional product information please contact the CytoCell Technical Support Department.
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REF	**EN:** Catalogue number
LOT	**EN**: Batch code
	EN: Consult instructions for use
	EN: Manufacturer
	EN: Use by
	EN: Temperature limitation
CONT	**EN:** Contents

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