



A Sysmex Group Company



Instructions For Use
REF: RU-LPU 026-S / RU-LPU 026

SRY Probe

Research Use Only

PROFESSIONAL USE ONLY

ENGLISH

Further information available at www.ogt.com

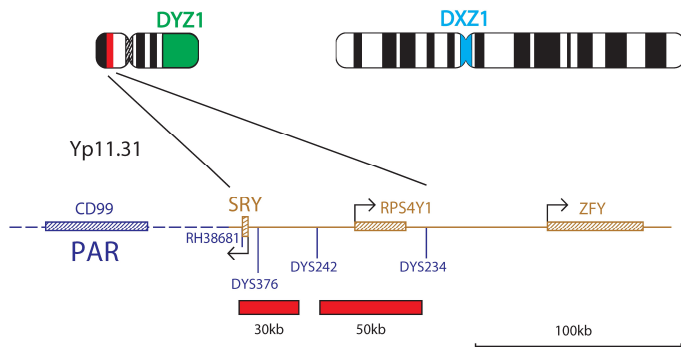
Fluorescence *In Situ* Hybridisation (FISH) is a technique that allows DNA sequences to be detected on metaphase chromosomes or in interphase nuclei from fixed cytogenetic samples. The technique uses DNA probes that hybridise to entire chromosomes or single unique sequences, and serves as a powerful 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

SRY, Yp11.31, Red
DYZ1, Yq12, Green
DXZ1, Xp11.1-q11.1, Blue



The SRY probe, labelled in red, consists of two non-overlapping probes, 30kb and 50kb. The probes cover the entire SRY gene and flanking DNA, including the RPS4Y1 gene. The probe mix also contains control probes for the X centromere (DXZ1), labelled in blue, and for chromosome Y (DYZ1, the heterochromatic block at Yq12), labelled in green.

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 -25°C to -15°C 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 80°C).
2. Variable volume micropipettes and tips range 1µl - 200µl.
3. Water bath with accurate temperature control at 72°C.
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. 37°C 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 37°C (+/- 1°C) 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 75°C (+/- 1°C) for 2 minutes.

Hybridisation

11. Place the slide in a humid, lightproof container at 37°C (+/- 1°C) 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 72°C (+/- 1°C) 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 male (XY) cell, there should be one red, one green and one blue signal (1R, 1G, 1B). A cell with a deletion in the region covered by the red clones in this probe set, which includes the SRY gene, should have one green and one blue signal (1G, 1B). In case of female (XX) genotype with male phenotype one red and two blue signals can be observed (1R, 2B). Other abnormal signal patterns may be seen.

Additional Information

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

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