



A Sysmex Group Company



REF: LPE 0XYc / LPE 0XYq

Dual Labelled Satellite Probe Sets

Research Use Only

Further information available at www.ogt.com

Intended Use

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

Probe Specification

XYc

DXZ1, Xp11.1- q11.1, Green

DYZ3, Yp11.1- q11.1, Red

XYq

DXZ1, Xp11.1- q11.1, Green

DYZ1, Yq12, Red

Chromosome X α -satellite (DXZ1) and Chromosome Y α -satellite (DYZ3)

This enumeration probe set contains chromosome specific alphoid DNA repeat sequences located at the centromeres of chromosome X and chromosome Y.

Chromosome X α -satellite (DXZ1) and Chromosome Y satellite III (DYZ1)

This enumeration probe set contains chromosome specific DNA repeat sequences located at the centromere of chromosome X and in the heterochromatic block of chromosome Y.

Materials Provided

Probe: 100 μ l per vial

The probes are provided in hybridisation solution (Formamide; Dextran Sulphate; SSC) and are ready to use. The probe DNA is directly labelled: X with a green fluorophore and Y probes with a red fluorophore.

Counterstain: 150 μ l per vial

The counterstain is DAPI antifade (ES: 0.125 μ g/ml DAPI (4,6-diamidino-2-phenylindole)).

Warnings and Precautions

1. For research use only. 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.

Protocol Recommendations

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.

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 1 μ l - 200 μ l.
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.

Sample Preparation

Samples should be prepared according to the laboratory or institution guidelines. Prepare air-dried samples on microscope slides according to slide preparation guidelines below.

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 10 μ l 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 10 μ l 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) for 1 hour.

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 10 μ l 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.

*If background is observed, slides can be washed through 0.25xSSC at step 13 instead of 0.4xSSC to increase stringency of the wash.

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.

Known Cross-Reactivity

The LPE 0XYc probe may show cross-hybridisation to chromosome Y and chromosome X and the centromeres of chromosomes 1, 11, 13, 14, 15, 17, 20, 21 and 22.

The LPE 0XYq probe may show cross-hybridisation to chromosome Y and the centromeres of chromosomes 1, 11 and 17.





Additional Information

For additional product information please contact the CytoCell Technical Support Department.

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REF	Catalogue number
LOT	Batch code
	Consult instructions for use
	Manufacturer
	Use by
	Temperature limitation
CONT	Contents

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