A Sysmex Group Company

CytoCell

REF: LPE 008B / LPE 012B / LPE 017B

Satellite Enumeration Probes

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
The probes are produced in a concentrated form to allow mixing, if required, of up to three probes in the same hybridisation, from CytoCell's range of concentrated Satellite probes. A final volume of 10µl of probe solution is required per hybridisation.
The probes are directly labelled with a blue fluorophore (Aqua or DEAC spectrum). For detailed probe specifications refer to Table 1.

Table 1: Probe Specifications

Chr	Catalogue Number*	Locus	Chromosome Region	DNA Class
8	LPE 008B	D8Z2	8p11.1-q11.1	α-satellite
12	LPE 012B	D12Z3	12p11.1-q11.1	α-satellite
17	LPE 017B	D17Z1	17p11.1-q11.1	α-satellite

***B specifies a blue label**

This kit contains only one of the probes from the range of directly labelled blue human alpha satellite probes.

Materials Provided
Probe: 30µl per vial
The probe is produced in a concentrated form. It is provided in hybridisation solution (Formamide; Dextran Sulphate; SSC).

Hybridisation solution (Formamide; Dextran Sulphate; SSC): 150µl per vial

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. 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 -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.

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 blue fluorophore has specificity to the Aqua and DEAC spectrum (single bandpass Aqua or DEAC filter is required).

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. Using fresh pipette tips remove (final volume of 10µl of probe solution):
   - for a **single probe hybridisation**: 3µl of probe and 7µl of hybridisation solution per test
   - for a **two probe hybridisation**: 3µl of each probe and 4µl of hybridisation solution per test
   - for a **three probe hybridisation**: 3µl of each probe and 1µl of hybridisation solution per test

   and transfer it to a microcentrifuge tube, gently vortex to mix and pulse spin in a microcentrifuge. Quickly return the remaining probe to -20ºC.
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) for 1 hour to overnight.

Post-Hybridisation Washes
12. Remove the coverslip and all traces of glue carefully.
13. Immerse the slide in 0.25xSSC (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.

* If final signal is poor, repeat FISH using 0.4xSSC post-hybridisation 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.

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
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Patents and Trademarks
CytoCell is a registered trademark of Cytocell Ltd.

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