



A Sysmex Group Company



REF: LPE xxxR/G

## Satellite Enumeration Probes

### Research Use Only

Further information available at [www.ogt.com](http://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 recommended per hybridisation.

The probes are directly labelled with either a red (Texas Red spectrum) or a green (FITC spectrum) fluorophore. For detailed probe specifications refer to Table 1.

**Table 1: Probe Specifications**

Chr	Catalogue Number*	Locus	Chromosome Region	DNA Class
1	LPE 001R/G	D1Z1	1q12	satellite III
2	LPE 002R/G	D2Z2	2p11.1-q11.1	α-satellite
3	LPE 003R/G	D3Z1	3p11.1-q11.1	α-satellite
4	LPE 004R/G	D4Z1	4p11.1-q11.1	α-satellite
1/5/19	LPE 005R/G	D1Z7 D5Z2 D19Z3	1p11.1-q11.1 5p11.1-q11.1 19p11.1-q11.1	α-satellite
6	LPE 006R/G	D6Z1	6p11.1-q11.1	α-satellite
7	LPE 007R/G	D7Z1	7p11.1-q11.1	α-satellite
8	LPE 008R/G	D8Z2	8p11.1-q11.1	α-satellite
9	LPE 009R/G	D9Z3	9q12	satellite III
10	LPE 010R/G	D10Z1	10p11.1-q11.1	α-satellite
11	LPE 011R/G	D11Z1	11p11.1-q11.1	α-satellite
12	LPE 012R/G	D12Z3	12p11.1-q11.1	α-satellite
13/21	LPE 013R/G	D13Z1 D21Z1	13p11.1-q11.1 21p11.1-q11.1	α-satellite
14/22	LPE 014R/G	D14Z1 D22Z1	14p11.1-q11.1 22p11.1-q11.1	α-satellite
15	LPE 015R/G	D15Z4	15p11.1-q11.1	α-satellite
16	LPE 016R/G	D16Z2	16p11.1-q11.1	α-satellite
17	LPE 017R/G	D17Z1	17p11.1-q11.1	α-satellite
18	LPE 018R/G	D18Z1	18p11.1-q11.1	α-satellite
20	LPE 020R/G	D20Z1	20p11.1-q11.1	α-satellite
X	LPE 0XR/G	DXZ1	Xp11.1-q11.1	α-satellite
Y	LPE 0YcR/G	DYZ3	Yp11.1-q11.1	α-satellite
Y	LPE 0YqR/G	DYZ1	Yq12	satellite III

\*R specifies a red label and G specifies a green label

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

#### Materials Provided

Probe: 15µl 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.
6. Ensure that the correct hybridisation times and SSC concentrations are used according to the protocol instructions provided for individual probes.

#### 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 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.

#### 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
8. and transfer it to a microcentrifuge tube, gently vortex to mix and pulse-spin in a microcentrifuge. 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) for 1 hour to overnight. For LPE 005R/G, LPE 016R and LPE 020R/G, 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 0.25xSSC (pH 7.0) at 72°C (+/- 1°C) for 2 minutes without agitation\*. For LPE 005R/G, LPE 016R and LPE 020R/G 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.

\*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.

#### Cross Reactivity for a single probe hybridisation

There will be differences in the relative size of signals observed between chromosomes due to the difference in copy number of repeat sequences between chromosomes.

1. Using one of the satellite probes for the chromosomes 1-12 and 15-20 (except 1/5/19 probe) a diploid sample should show a fluorescent signal at the centromere of both of the corresponding chromosomes.
2. Using the probe for chromosomes 1/5/19 a diploid sample should show a fluorescent signal at the centromere of each of the chromosomes 1, 5 and 19.
3. Using the probe for chromosomes 13/21 or 14/22 a diploid sample should show a fluorescent signal at the centromere of both of the chromosomes for chromosomes 13 and 21 or 14 and 22.
4. The chromosome 1 satellite III probe may show faint cross-hybridisation to the pericentromeric region of chromosome 9. This may be reduced when using a 0.25xSSC stringent wash, compared to a 0.4xSSC stringent wash.
5. The chromosome 2  $\alpha$ -satellite probe may show faint cross-hybridisation to the centromere of an F group chromosome. This may be reduced when using a 0.25xSSC stringent wash, compared to a 0.4xSSC stringent wash.
6. The chromosome 4  $\alpha$ -satellite probe may show faint cross-hybridisation to the centromeric region of a C group chromosome. This may be reduced when using a 0.25xSSC stringent wash, compared to a 0.4xSSC stringent wash.






#### Additional Information

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

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