A Sysmex Group Company

Cytocell

REF: LPT xxxR/G

Subtelomere Specific Probes

Research Use Only

Further information available at www.cytocell.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 subtelomere specific probe range identifies 41 of the 46 human telomeres as it excludes the p-arm telomeres of the acrocentric chromosomes. The X and Y chromosome p-arms share the same subtelomere clone (839D20) as do the X and Y q-arms (C8.2/1 and 225F6) due to the pseudoautosomal nature of these regions. The probes are directly labelled with either a red or a green fluorophore. For detailed probe specifications refer to Table 1.

Table 1: Probe Specifications

Probe	Catalogue Number	Clone name	Marker	Accession Number (if available)
1p	LPT 01PR/G	CEB108	RH120573	-
1q	LPT 01QR/G	160H23	GDB:315525	D1S3739
2p	LPT 02PR/G	dJ892G20	D2S2983	D2S2983
2q NP	LPT 02QNPR/G	172I13	D2S447	D2S2986
3p	LPT 03PR/G	dJ1186B18	D3S4559	D3S4559
3q	LPT 03QR/G	196F4	D3S1272	D3S1272
4p	LPT 04PR/G	36P21	D4S3360	D4S3360
4q	LPT 04QR/G	963K6	D4S139	-
5p	LPT 05PR/G	189N21	RH120167	-
5q	LPT 05QR/G	240G13	D5S2907	D5S2907
6p	LPT 06PR/G	62I11	STS-H99640	-
6q	LPT 06QR/G	57H24	D6S2522	D6S2522
7p	LPT 07PR/G	109a6	RH104000	RH104000
7q	LPT 07QR/G	2000a5	RH48601	RH48601
8p	LPT 08PR/G	dJ580L5	RH40619	D8S2333
8q	LPT 08QR/G	489D14	D8S595	D8S1925
9p	LPT 09PR/G	43N6	RH65569	RH65569
9q	LPT 09QR/G	112N13	D9S2168	D9S2168
10p	LPT 10PR/G	306F7	STS-N35887	D10S2488
10q	LPT 10QR/G	137E24	RH44494	RH44494
11p	LPT 11PR/G	dJ908H22	D11S2071	D11S2071
11q	LPT 11QR/G	dJ770G7	D11S4974	D11S4974
12p	LPT 12PR/G	496A11	D12S200	D12S200
12q	LPT 12QR/G	221K18	RH81094	D12S2343
13q	LPT 13QR/G	163C9	D13S1825	D13S1825
14q	LPT 14QR/G	dJ820M16	D14S1420	D14S1420
15q	LPT 15QR/G	154P1	D15S936	D15S936
16p	LPT 16PR/G	121I4	SHGC-16929(UCSC)	D16S3400
16q	LPT 16QR/G	240G10	RH80305	RH80305
17p	LPT 17PR/G	202L17 2111b1	D17S2199	D17S2199
17q	LPT 17QR/G	362K4	362K4 For and Rev	D17S2200
18p	LPT 18PR/G	74G18	D18S552	D18S552
18q	LPT 18QR/G	dJ964M9	D18S1390	D18S1390
19p	LPT 19PR/G	dJ546C11	D19S676E	-
19q	LPT 19QR/G	F21283	RH102404	RH102404
20p	LPT 20PR/G	dJ1061L1	D20S210	D20S502
20q	LPT 20QR/G	81F12	RH10656	-
21q	LPT 21QR/G	63H24	D21S1446	D21S1575
22q	LPT 22QR/G	99K24 N85a3	D22S1726	D22S1726
XpYp **	LPT XYPR/G	839D20	DXYS129	DXYS129
XqYq ***	LPT XYQR/G	225F6 C8.2/1	DXYS154 SYBL1	Z43206 -

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

**This probe is specific for the p-arms of both X and Y.
***This probe is specific for q-arms of both X and Y.

This Aquarius® kit contains only one of the probes from the range of directly labelled subtelomere specific probes.

Materials Provided
Probe: 15µl per vial
The probe is produced in a concentrated form. It is labelled with either a red or a green fluorophore. The probe 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. Users of this product must be capable of visually distinguishing between the colours red, blue and green.

Storage and Handling
The Aquarius® 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 fluorescence microscope should be checked before use to ensure it is operating correctly. Immersion oil should be suitable for use in fluorescence microscopy and formulated for low autofluorescence. Manufacturers' recommendations should be followed in regards to the life of the lamp and the age of the filters.

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. Briefly centrifuge tubes before use
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. 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.

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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