A Sysmex Group Company

CytoCell

Instructions For Use
REF: LPE NOR-S / LPE NOR

Acro-P-Arm Probe

Research Use Only

PROFESSIONAL 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
NOR (Nucleolar Organizer Regions) probe is specific for rRNA genes located in the short arms of the acrocentric chromosomes (13, 14, 15, 21 and 22), labelled in red.

Materials Provided
Probe: 50µl per vial or 100µl per vial
The probe is provided premixed in hybridisation solution (Formamide; Dextran Sulphate; SSC) and is 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. Dispose of all hazardous materials according to your institution's guidelines for hazardous waste disposal.
6. Operators must be capable of visually distinguishing between red, blue and green.

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.

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
Use a 100-watt mercury lamp and plan apochromat objectives x63 or x100 for optimal visualisation. Use a triple bandpass filter DAPI/FITC/Texas Red for optimal visualisation of all fluorophores and DAPI simultaneously.
Check the fluorescence microscope before use to ensure it is operating correctly. Use immersion oil that is suitable for fluorescence microscopy and formulated for low autofluorescence. Follow manufacturers' recommendations in regards to the life of the lamp and the age of the filters.

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: 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 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. Use of a calibrated thermometer 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
Up to ten signals, which vary in size.

Known Cross-Reactivity
No known cross-reactivity.

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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