A Sysmex Group Company

CytoCell

REF: RU-LPS 038-S / RU-LPS 038

IGK Breakapart Probe

Research Use Only

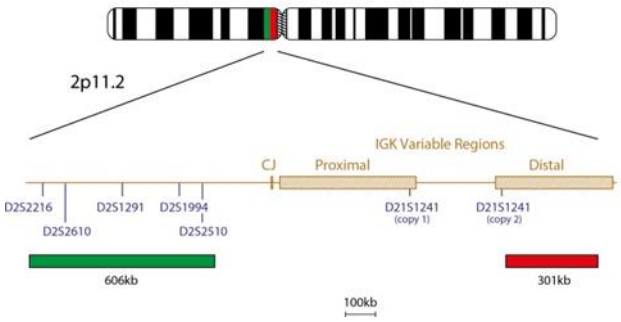
Further information available at www.ogt.com

Fluorescence *In Situ* Hybridisation (FISH) is a technique that allows the visualisation of DNA sequences upon chromosomes. The technique uses DNA probes that hybridise to entire chromosomes or single unique sequences, and serves as a powerful adjunct to classic cytogenetics. Recent developments have meant that this valuable technique can now be applied as an essential tool in prenatal, haematological and pathological chromosomal analysis. Target DNA, after fixation and denaturation, is available for annealing to a similarly denatured, fluorescently labelled DNA probe, which has a complementary sequence. Following hybridisation, unbound and non-specifically bound DNA probe is removed and the DNA is counterstained for visualisation. Fluorescence microscopy then allows the visualisation of the hybridised probe on the target material.

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

Probe Specification
IGK, 2p11.2, Red
IGK, 2p11.2, Green

CMP-H034 v005.00



The IGK product consists of a 301kb probe, labelled in red, covering a part of the distal IGK Variable region and a green probe, covering a 606kb region telomeric to the Joining segments and the Constant segment of IGK. The green probe extends from a position that is telomeric to the D2S2216 marker and continues to a position that is centromeric to the D2S2510 marker.

Materials Provided
Probe: 50µl per vial or 100µl per vial
The probes are provided premixed in hybridisation solution (Formamide; Dextran Sulphate; SSC) and are 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. All hazardous materials should be disposed of according to your institution's guidelines for hazardous waste disposal.

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
For use on Formalin Fixed Paraffin Embedded (FFPE) tissue sections, Tissue Microarrays (TMA), peripheral blood samples or cultured bone marrow cells.

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.
15. Tissue Pretreatment Kit (LPS 100).

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. Alternatively for viewing red and green fluorophores use dual bandpass filter FITC/Texas Red. 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. For tissue FISH, 4µm - 6µm thick FFPE tissue sections should be used.

FISH Protocol
(Note: Please ensure that exposure of the probe to laboratory lights is limited at all times)

FFPE Procedure
Tissue Sample Pretreatment
Tissue sample pretreatment should be done according to the laboratory or institution guideline. For optimal results use the Tissue Pretreatment Kit (LPS 100).

Pre-Denaturation
1. Remove the probe from the freezer and allow it to warm to room temperature (RT)
2. Ensure that the probe solution is uniformly mixed with a pipette.
3. Remove 10µl - 15µl (depending on the size of the tissue) of probe per test, and transfer it to a microcentrifuge tube. Quickly return the remaining probe to the freezer.
4. Place the probe and the sample slide to prewarm on a 37ºC (+/- 1ºC) hotplate for 5 minutes.
5. Spot 10µl - 15µl of probe mixture onto the sample and carefully apply a coverslip. Seal with rubber solution glue and allow the glue to dry completely.

Denaturation
6. Denature the sample and probe simultaneously by heating the slide on a hotplate at 75ºC (+/- 1ºC) for 5 minutes.

Hybridisation
7. Place the slide in a humid, lightproof container at 37ºC (+/- 1ºC) overnight.

Post-Hybridisation Washes
8. Remove the coverslip and all traces of glue carefully.
9. Immerse the slide in 0.4xSSC (pH 7.0) at 72ºC (+/- 1ºC) for 2 minutes without agitation.
10. Drain the slide and immerse it in 2xSSC, 0.05% Tween-20 at RT (pH 7.0) for 30 seconds without agitation.
11. Drain the slide and apply 10µl - 15µl of DAPI antifade onto each sample.
12. Cover with a coverslip, remove any bubbles and allow the colour to develop in the dark for 10 minutes.
13. View with a fluorescence microscope.

Comments
Hybridisation efficiency and tissue morphology are usually negatively correlated. Aggressive pretreatment procedures improving hybridisation efficiency (e.g. an extended enzyme digestion time) tend to destroy cell structure and tissue morphology. However, mild pretreatment saving tissue structures may not be sufficient for probe penetration and successful FISH results.
The optimal length of heat pretreatment and enzyme digestion time will depend on the age of the block, the tissue composition, and quality of tissue fixation. Enzyme digestion should be decreased for core biopsies and any sections that contain few tumour cells or have large areas of necrosis. These samples need to be handled particularly carefully to avoid over-digestion.

Peripheral blood or cultured bone marrow Procedure
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 -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) 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 - 15µl 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 below 4ºC.

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.

Expected Results

In the normal cell, 2 red/green (can appear as yellow, Y) signals are expected (2Y). A translocation will result in 1R, 1G, 1Y: 1 green and 1 red signal from the translocated chromosomes and 1 fused red/green signals (can appear as yellow) from the normal chromosome.

Additional Information

For additional product information please contact the CytoCell Technical Support Department.
T: +44 (0)1223 294048
E: techsupport@cytocell.com
W: www.ogt.com

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

Patents and Trademarks

CytoCell is a registered trademark of Cytocell Ltd.
This product contains technology licensed from Life Technologies Corporation and is available for research use only.

Cytocell Ltd.
Oxford Gene Technology,
418 Cambridge Science Park,
Milton Road,
Cambridge, CB4 0PZ, UK
T: +44(0)1223 294048
F: +44(0)1223 294986
E: probes@cytocell.com
W: www.ogt.com