Instructions For Use
REF: AMPxxR/G

Murine Whole Chromosome Painting Probes

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Further information available at www.cytocell.com

Fluorescence *In Situ* Hybridisation (FISH) is a technique that allows DNA sequences to be detected on metaphase chromosomes or in interphase nuclei from fixed cytogenetic samples. The technique uses DNA probes that hybridise to entire chromosomes or single unique sequences, and serves as a powerful adjunct to classic cytogenetics. 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.

Probe Information
Whole chromosome painting probes are specific for each chromosome and are derived from flow-sorted chromosomes. They are libraries of many sequential sequences extending along the whole length of the chromosome.

Probe Specification
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

Chromosome	Catalogue Number – 5 test*	Catalogue Number – 10 test*
1	AMP01R/G-S	AMP01R/G
2	AMP02R/G-S	AMP02R/G
3	AMP03R/G-S	AMP03R/G
4	AMP04R/G-S	AMP04R/G
5	AMP05R/G-S	AMP05R/G
6	AMP06R/G-S	AMP06R/G
7	AMP07R/G-S	AMP07R/G
8	AMP08R/G-S	AMP08R/G
9	AMP09R/G-S	AMP09R/G
10	AMP10R/G-S	AMP10R/G
11	AMP11R/G-S	AMP11R/G
12	AMP12R/G-S	AMP12R/G
13	AMP13R/G-S	AMP13R/G
14	AMP14R/G-S	AMP14R/G
15	AMP15R/G-S	AMP15R/G
16	AMP16R/G-S	AMP16R/G
17	AMP17R/G-S	AMP17R/G
18	AMP18R/G-S	AMP18R/G
19	AMP19R/G-S	AMP19R/G
X	AMP0XR/G-S	AMP0XR/G
Y	AMP0YR/G-S	AMP0YR/G

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

This Aquarius® kit contains only one of the probes from the range of directly labelled whole chromosome painting probes.

Materials Provided
Probe: 50µl or 100 µl per vial (5 or 10 tests)
Amount of red whole chromosome painting probe: 12-25ng/test
Amount of green whole chromosome painting probe: 56-94ng/test
The probes are provided in hybridisation solution (Formamide; Dextran Sulphate; SSC) and are ready to use. They are labelled with either a red or a green fluorophore.

Counterstain: 150µl per vial (15 tests)
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.
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 Aquarius® kit should be stored at -20ºC 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 Triple bandpass filter DAPI/FITC/Texas Red is optimal for viewing all fluorophores and DAPI simultaneously.

Sample Preparation
The kit has been designed for use on:
- Formalin Fixed Paraffin Embedded (FFPE) Tissue sections or Tissue Microarrays (TMA), 4µm - 6µm thick tissue sections should be used.
- Cultured cells fixed in Carnoy's fixative.

All samples should be prepared according to the laboratory or institution guidelines.

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 Aquarius® 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 15µl of probe per test, and transfer it to a microcentrifuge tube. Quickly return the remaining probe to -20ºC.
4. Place the probe and the sample slide to prewarm on a 37ºC (+/- 1ºC) hotplate for 5 minutes.
5. Spot 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 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.

Procedure for Fixed Cell Suspensions

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 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) for 4 hours to 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.

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.

Suggestions for mixing probes

1. Mixing one red probe and one green probe: 5µl of red probe and 5µl of green probe per test for fixed cell suspensions and 7.5µl of red probe and 7.5µl of green probe per test for FFPE tissue.

These volumes are for use as recommendations only. These volumes may require optimisation.

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. 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

1. Centromeres are not labelled by these paints.
2. Red paints may sometimes give a general speckly "loose association" (rather than a clear cross-hybridisation) with other chromosomes. This is nowhere near as bright as the painted chromosome, which can be clearly distinguished.
3. Chromosome 1 paint may give occasional telomeric–like signals on one to two other chromosomes.
4. Chromosome 10 may show a slight painting effect with one other chromosome (X or Y).
5. Chromosome 13 may show a slight painting effect with two other chromosomes.
6. Chromosomes 15,16,17,18 and 19 paints may show a slight painting effect with one other chromosome (X or Y)
7. Chromosome X paint also labels two sections on the Y chromosome (expected homologous regions).
8. Chromosome Y paint also gives signals on chromosome X (expected homologous regions).

Limitations

For Research Use Only. Not for use in diagnostic procedures. This product has only been tested on FFPE sections and fixed cell suspensions from mouse.

Additional Information

For additional product information please contact the Cytocell Technical Support Department.
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REF	**EN:** Catalogue number
LOT	**EN**: Batch code
	**EN:** Consult instructions for use
	**EN:** Manufacturer
	**EN:** Use by
	**EN:** Temperature limitation
	**EN:** Sufficient for <n> tests
CONT	**EN:** Contents

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