A Sysmex Group Company

CytoCell

Instructions For Use

myProbes

(MPD product codes)

Research Use Only

PROFESSIONAL USE ONLY

Further information available at www.ogt.com

Principles of the Test

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 G-banded cytogenetic analysis. This technique can now be applied as an essential investigative tool within prenatal, haematological and solid tumour 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.

Materials Provided

Probe: 100µl per vial
The probes are provided premixed in hybridisation solution (formamide; dextran sulfate; saline-sodium citrate (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) in glycerol-based mounting medium).

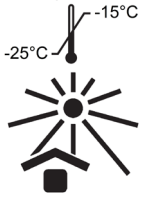
Warnings and Precautions

1. For research use only. Not for use in diagnostic procedures.
2. For laboratory professional use only.
3. Probe mixtures contain formamide, which is a teratogen; do not breathe fumes or allow skin contact. Handle with care; wear gloves and a lab coat.
4. DAPI is a potential carcinogen. Handle with care; wear gloves and a lab coat.
5. Follow local disposal regulations for your location along with recommendations in the Safety Data Sheet to determine the safe disposal of this product. This also applies to damaged test kit contents.
6. Dispose of all used reagents and any other contaminated disposable materials following procedures for infectious or potentially infectious waste. It is the responsibility of each laboratory to handle solid and liquid waste according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.
7. Operators must be capable of distinguishing the colours red, blue, and green.
8. Failure to adhere to the outlined protocol and reagents may affect the performance and lead to false positive/negative results.
9. The probe should not be diluted or mixed with other probes.
10. Failure to use the correct volume of probe during the pre-denaturation stage of the protocol may affect the performance and lead to false positive/negative results.
11. All products should be validated before use.
12. Internal controls should be carried out by using unaffected cell populations in testing samples.

Temperature Definitions

- -20°C / Frozen / In the Freezer: -25°C to -15°C
- 37°C: +37°C ± 1°C
- 72°C: +72°C ± 1°C
- 75°C: +75°C ± 1°C
- Room Temperature (RT): +15°C to +25°C

Storage and Handling



The kit should be stored between -25ºC to -15ºC in a freezer. The probe and counterstain vials must be stored in the dark.

Exposure to light should be minimised and avoided wherever possible. Store components in the light proof container provided. Components used and stored under conditions other than those stated on the labelling may not perform as expected and may adversely affect the assay results. All efforts must be made to limit exposure to light and temperature changes.

Equipment and Materials Necessary but not Supplied

Calibrated equipment must be used:

1. Hotplate (with a solid plate and accurate temperature control up to 80ºC)
2. Calibrated variable volume micropipettes and tips range 1µl - 200µl
3. Water bath with accurate temperature control at 37ºC and 72ºC
4. Microcentrifuge tubes (0.5ml)
5. Fluorescence microscope (Please see Fluorescence Microscope Recommendation section)
6. Phase contrast microscope
7. Clean plastic, ceramic or heat-resistant glass Coplin jars
8. Forceps
9. Calibrated pH meter (or pH indicator strips capable of measuring pH 6.5 – 8.0)
10. Humidified container
11. Fluorescence grade microscope lens immersion oil
12. Bench top centrifuge
13. Microscope slides
14. 24x24mm coverslips
15. Timer
16. 37ºC incubator
17. Rubber solution glue
18. Vortex mixer
19. Graduated cylinders
20. Magnetic stirrer
21. Calibrated thermometer

Optional Equipment not Supplied

1. Cytogenetic drying chamber

Reagents Needed but not Supplied

1. CytoCell Tissue Pretreatment Kit (LPS 100)
2. 20x saline-sodium citrate (SSC) Solution
3. 100% Ethanol
4. Tween-20
5. 1M Sodium hydroxide (NaOH)
6. 1M Hydrochloric acid (HCl)
7. Purified water

Fluorescence Microscope Recommendation

Use a 100-watt mercury lamp or equivalent and oil immersion plan apochromat objectives 60/63x or 100x for optimal visualisation. The fluorophores used in our probe sets will excite and emit at the following wavelengths:

Fluorophore	$Excitation_{max}$ [nm]	$Emission_{max}$ [nm]
DAPI	364	454
Aqua	418	467
Green	495	521
Red	596	615
Gold	539	561
Orange	551	572

Ensure appropriate excitation and emission filters that cover the relevant wavelengths listed above are fitted to the microscope.
Use a triple bandpass DAPI/green spectrum/red spectrum filter or a dual bandpass green spectrum/red spectrum filter for optimal simultaneous visualisation of the green and red fluorophores.
Use a single bandpass aqua spectrum filter for optimal visualisation of the aqua spectrum or a triple bandpass red spectrum/green spectrum/aqua spectrum filter for simultaneous visualisation of the green, red and aqua fluorophores.

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 auto fluorescence. Avoid mixing DAPI antifade with microscope immersion oil as this will obscure signals. Follow manufacturers' recommendations in regards to the life of the lamp and the age of the filters.

Sample Preparation

The kit is designed for use on haematologically derived cell suspensions fixed in Carnoy's solution (3:1 methanol/acetic acid) fixative, and for use on Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections, that are prepared according to the laboratory or institution guidelines. Prepare air dried haematologically derived samples, or FFPE tissue sections, on microscope slides according to standard cytogenetic procedures.

Tissue Sample Pretreatment (FFPE samples only)

Tissue sample pretreatment should be done according to the laboratory or institution guidelines. Use the CytoCell Tissue Pretreatment Kit (LPS 100) for optimal results.

FISH Protocol
(Note: Ensure that exposure of the probe and counterstain to laboratory lights is limited at all times).

***Slide Preparation* (haematologically derived samples only)**
1. Spot the cell sample onto a glass microscope slide. Allow to dry. (**Optional, if using a cytogenetic drying chamber,** the chamber should be operated at approximately 25°C and 50% humidity for optimal cell sample spotting. If a cytogenetic drying chamber is not available, use a fume hood as an alternative).
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. Remove 10µl of probe per test, and transfer it to a microcentrifuge tube. Quickly return the remaining probe to the freezer. (Use 10µl to 15µl of probe for FFPE samples.)
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. (Use 10µl to 15µl of probe for FFPE samples.)

Denaturation
10. Denature the sample and probe simultaneously by heating the slide on a hotplate at 75ºC (+/- 1ºC) for 2 minutes (haematologically derived samples only) or at 75ºC (+/- 1ºC) for 5 minutes (FFPE samples only).

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

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

Procedural Recommendations
1. Baking or ageing of slides 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 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.
6. Over hybridisation can result in additional or unexpected signals.
7. Users should optimise the protocol for their own samples.
8. Suboptimal conditions may result in non-specific binding that may be misinterpreted as a probe signal.

Additional Information
For additional product information please contact the CytoCell Technical Support Department.
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Symbols Glossary

ISO 15223-1:2016 - "Medical devices - Symbols to be used with medical device labels, labelling and information to be supplied - Part 1: General requirements" (© International Organization for Standardization)		
Symbol	**Title**	**Reference Number(s)**
	**en:** Manufacturer	5.1.1
	**en**: Date of manufacture	5.1.3
	**en**: Batch code	5.1.5
	**en:** Catalogue number	5.1.6
	**en:** Keep away from sunlight	5.3.2
	**en:** Temperature limit	5.3.7
	**en:** Consult instructions for use	5.4.3
	**en:** Caution	5.4.4

EDMA symbols for IVD reagents and components, October 2009 revision		
Symbol	**Title**	**Reference Number(s)**
	**en:** Contents (or contains)	N/A

Patents and Trademarks
CytoCell is a registered trademark of Cytocell Limited.

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V001 2021-05-25: New IFU.