



A Sysmex Group Company



Instructions for Use

REF: RU-LPH 095 / RU-LPH 095-S

Del(5q) Plus Tri-Colour Deletion Probe

Research Use Only

PROFESSIONAL USE ONLY

Further information available at www.ogt.com/cytozell

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.

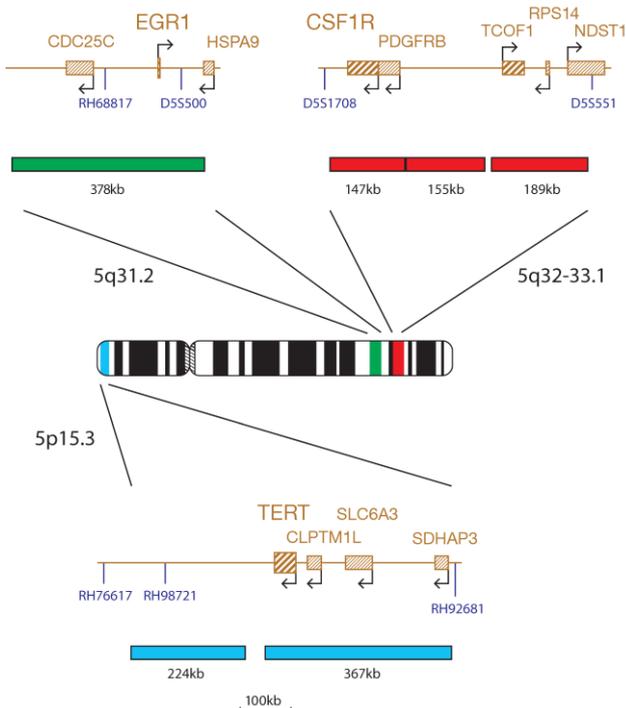
Intended Use

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

Probe Specification

TERT, 5p15.3, Aqua
EGR1, 5q31.2, Green
CSF1R, 5q32-33.1, Red

CMP-H122 v001.00



The Del(5q) Plus Tri-Colour Deletion Probe mix consists of three distinct probes. The green probe (378kb) covers the CDC25C and EGR1 genes, along with their flanking regions that include the RH68817 and D5S500 markers. The red probe set (147kb, 155kb and 189kb) locates between the D5S1708 and D5S551 markers and includes the CSF1R, PDGFRB, TCOF1 and RPS14 genes. The aqua probe set

(224kb and 367kb) locates between the markers RH76617 and RH92681 and includes the genes TERT, CLPTM1L, SLC6A3 and SDHAP3.

Materials Provided

Probe: 50µl per vial (5 tests) or 100µl per vial (10 tests)
The probes are provided premixed in hybridisation solution (formamide; dextran sulfate; saline-sodium citrate (SSC)) and are ready to use.

Counterstain: 150µl per vial (15 tests)

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 10µl 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

-15°C The kit should be stored between -25°C to -15°C in a freezer until the expiry date indicated on the kit label. The probe and counterstain vials must be stored in the dark.

The FISH probe, DAPI Antifade ES counterstain and Hybridisation Solution remain stable throughout the freeze-thaw cycles experienced during normal use (where one cycle constitutes the vial's removal from and replacement into the freezer). 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 80°C)
 2. Calibrated variable volume micropipettes and tips range 1µl - 200µl
 3. Water bath with accurate temperature control at 37°C and 72°C
 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. 37°C 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. 20x saline-sodium citrate (SSC) Solution
2. 100% Ethanol

3. Tween-20
4. 1M Sodium hydroxide (NaOH)
5. 1M Hydrochloric acid (HCl)
6. 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 this probe set will excite and emit at the following wavelengths:

Fluorophore	Excitation _{max} [nm]	Emission _{max} [nm]
Aqua	418	467
Green	495	521
Red	596	615

Ensure appropriate excitation and emission filters that cover the wavelengths listed above are fitted to the microscope. 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 that are prepared according to the laboratory or institution guidelines. Prepare air dried samples on microscope slides according to standard cytogenetic procedures. The AGT *Cytogenetics Laboratory Manual* contains recommendations for specimen collection, culturing, harvesting and for slide making¹.

Solution Preparation

Ethanol Solutions

Dilute 100% ethanol with purified water using the following ratios and mix thoroughly:

- 70% Ethanol - 7 parts 100% ethanol to 3 parts purified water
- 85% Ethanol - 8.5 parts 100% ethanol to 1.5 parts purified water

Store the solutions for up to 6 months at room temperature in an airtight container.

2xSSC Solution

Dilute 1 part 20xSSC Solution with 9 parts purified water and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

0.4xSSC Solution

Dilute 1 part 20xSSC Solution with 49 parts purified water and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

2xSSC, 0.05% Tween-20 Solution

Dilute 1 part 20xSSC Solution with 9 parts purified water. Add 5µl of Tween-20 per 10ml and mix thoroughly. Check pH and adjust to pH 7.0 using NaOH or HCl as required. Store the solution for up to 4 weeks at room temperature in an airtight container.

FISH Protocol

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

Slide Preparation

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.
8. Place the probe and the sample slide to prewarm on a 37°C (+/- 1°C) 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 75°C (+/- 1°C) for 2 minutes.

Hybridisation

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

Post-Hybridisation Washes

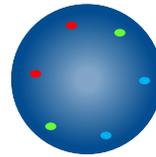
12. Remove the DAPI from the freezer and allow it to warm to RT.
13. Remove the coverslip and all traces of glue carefully.
14. Immerse the slide in 0.4xSSC (pH 7.0) at 72°C (+/- 1°C) for 2 minutes without agitation.
15. Drain the slide and immerse it in 2xSSC, 0.05% Tween-20 at RT (pH 7.0) for 30 seconds 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.

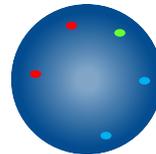
Expected Results

Expected Normal Signal Pattern

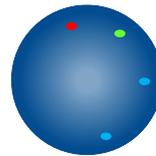


In a normal cell, two aqua, two green and two red signals are expected (2A2G2R).

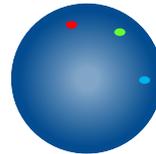
Expected Abnormal Signal Patterns



In a cell with a hemizygous deletion of 5q31.2, the expected signal pattern will be two aqua, one green and two red signals (2A1G2R).



In a cell with a hemizygous deletion of 5q, the expected signal pattern will be two aqua, one green and one red signal (2A1G1R).



In a cell with monosomy 5, the expected signal pattern will be one aqua, one green and one red signal (1A1G1R).

Other signal patterns are possible in aneuploid/unbalanced specimens.

Known Relevant Interferences / Interfering Substances

No known relevant interferences / interfering substances.

Known Cross-Reactivity

No known cross-reactivity.

Additional Information

For additional product information please contact the CytoCell Technical Support Department.

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References

1. Arsham MS, Barch MJ and Lawce HJ. (eds.) (2017) *The AGT Cytogenetics Laboratory Manual*. New Jersey: John Wiley & Sons Inc.

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: Use-by date	5.1.4
	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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IFU Version History

V001.00 2021-10-01: Creation of IFU for new product