A Sysmex Group Company

CytoCell

**Instructions For Use**
**REF: RU-LPH 012-S / RU-LPH 012**

# TEL/AML1 Translocation, Dual Fusion Probe

## Research Use Only

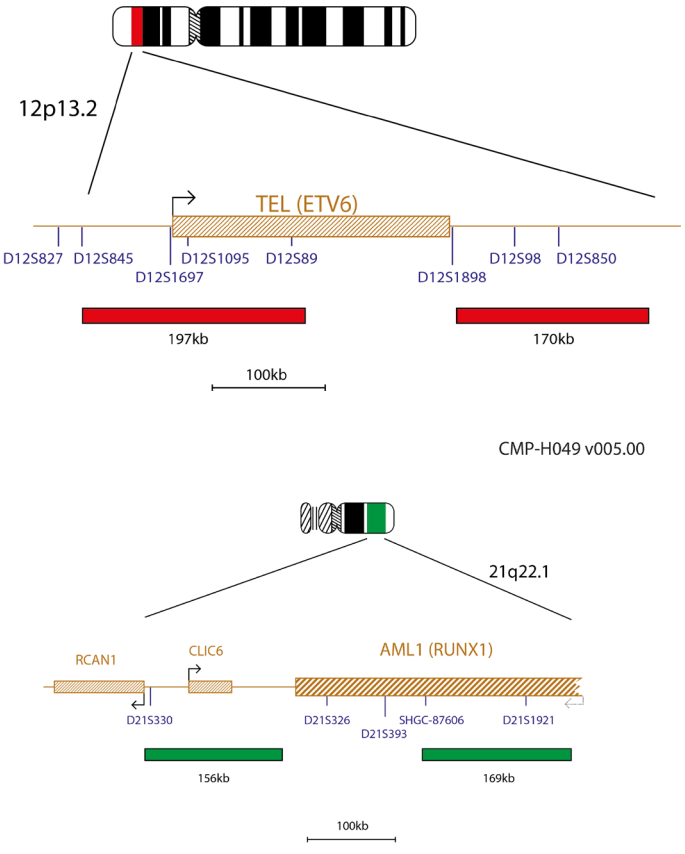
**PROFESSIONAL USE ONLY**

**Further information available at www.ogt.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. 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**
TEL (ETV6), 12p13.2, Red
AML1 (RUNX1), 21q22.1, Green




The TEL probe mix, labelled in red, contains a probe covering a 197kb region at the telomeric end of TEL (ETV6) and a second probe that extends 170kb in a centromeric direction beyond the TEL (ETV6) gene. The AML1 (RUNX1) probe set consists of two probes labelled in green. One locates to a 156kb region centromeric to the AML1 (RUNX1) gene and covers the CLIC6 gene. The other probe covers a 169kb region in the AML1 (RUNX1) gene that includes the markers SHGC-87606 and D21S1921.

**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**

**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**
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: Please 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. 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 a normal cell these probes should appear as discrete red and green spots, one for each homologue (resulting in a 2R, 2G conformation). In a t(12;21)(p13.2;q22.12) cell there should be two yellow fusion signals in addition to the red and green signals of the normal chromosomes 12 and 21 respectively (1R, 1G, 2Y).

**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

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| **REF** | **EN:** Catalogue number |
| LOT | **EN**: Batch code |
|  | **EN:** Consult instructions for use |
|  | **EN:** Manufacturer |
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|  | **EN:** Temperature limitation |
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**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