



A Sysmex Group Company



Instructions For Use

REF: LPH 027-S / LPH 027

AML1 (RUNX1) Breakapart Probe



PROFESSIONAL USE ONLY



www.cytoCELL.com

Further information and other languages available at www.ogt.com

Limitations

This device is designed to detect rearrangements with breakpoints in the region bounded by the red and green clones in this probe set, which includes the *AML1* (*RUNX1*) region. Breakpoints outside this region or variant rearrangements wholly contained within this region may not be detected with this product.

Due to the scattered breakpoint region, in some rearrangements in acute lymphoblastic leukaemia (ALL) users may observe variant signal patterns.

The test is not intended for: use as a stand-alone diagnostic, prenatal testing, population-based screening, near-patient testing or self-testing. This product is intended for laboratory professional use only; all results should be interpreted by suitably-qualified staff, taking into account other relevant test results.

This product has not been validated for use on sample types or disease types other than those specified in the intended use.

Reporting and interpretation of FISH results should be consistent with professional standards of practice and should take into consideration other clinical and diagnostic information. This kit is intended as an adjunct to other diagnostic laboratory tests and therapeutic action should not be initiated on the basis of the FISH result alone.

Failure to adhere to the protocol may affect the performance and lead to false positive/negative results.

This kit has not been validated for purposes outside of the intended use stated.

Intended Use

The CytoCell AML1 (RUNX1) Breakapart Probe is a qualitative, non-automated, fluorescence *in situ* hybridisation (FISH) test used to detect chromosomal rearrangements in the 21q22.1 region on chromosome 21 in Carnoy's solution (3:1 methanol/acetic acid) fixed haematologically-derived cell suspensions from patients with confirmed or suspected acute myeloid leukaemia (AML) or acute lymphoblastic leukaemia (ALL).

Indications

This product is designed as an adjunct to other clinical and histopathological tests in recognised diagnostic and clinical care pathways, where knowledge of *AML1* (*RUNX1*) rearrangement status would be important for clinical management.

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.

Probe Information

The *RUNX1* (*RUNX* family transcription factor 1) gene at 21q22.1 is one of the most frequent targets of chromosomal rearrangements observed in human acute leukaemia.

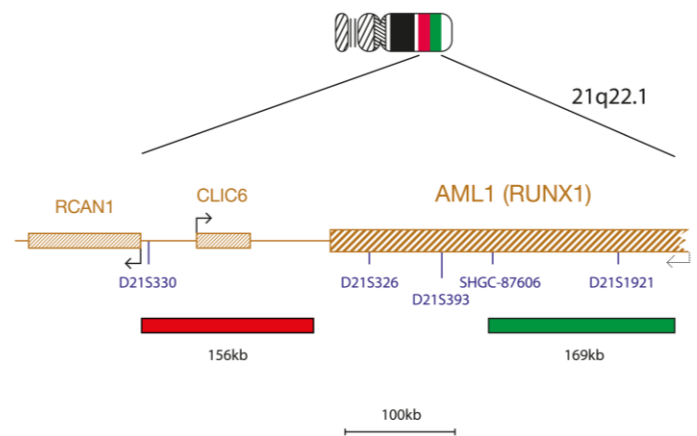
The most common rearrangements are the ETV6-*RUNX1* and *RUNX1*-*RUNX1T1* fusions. The ETV6-*RUNX1* fusion is brought about by the t(12;21)(p13;q22) translocation, observed in around 21% of childhood B-cell acute lymphoblastic leukaemia (ALL) cases¹, whilst the *RUNX1*-*RUNX1T1* fusion is the result of the t(8;21)(q22;q22) translocation observed in 10-22% of patients with acute myeloid leukaemia (AML) FAB (French-American-British classification) type M2 and 5-10% of AML cases overall^{2,3}. Both these rearrangements are considered good prognostic indicators in these diseases^{4,5}.

The *RUNX1* gene is also rearranged in many other rarer translocations, with partners including chromosomes 1, 2, 3, 4, 5, 6, 7, 9, 10, 14, 15, 16, 17, 18, 19, 20 and X⁶. This breakapart probe has been designed to allow the detection of rearrangements regardless of the partner gene.

Rearrangements of *RUNX1* are not restricted to translocations. Using FISH, amplifications of chromosome 21 (iAMP21), including the *RUNX1* gene, have also been found in childhood ALL^{7,8}. These amplifications have been associated with poorer outcome⁹.

Probe Specification

AML1, 21q22.1, Red
AML1, 21q22.1, Green



The AML1 probe mix consists of a 156kb probe, labelled in red, centromeric to the AML1 (*RUNX1*) gene that spans the *CLIC6* gene and a 169kb probe, labelled in green, covering part of the AML1 (*RUNX1*) gene, including markers SHGC-87606 and D21S1921.

Materials Provided

Probe: 50µl per vial (5 tests) or 100µl per vial (10 tests)

The probes are provided premixed in hybridisation solution (formamide; dextran sulphate; 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)).

Warnings and Precautions

1. For *in vitro* diagnostic use. 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. 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. Dispose of all hazardous materials according to your institution's guidelines for hazardous waste disposal.
6. Operators must be capable of distinguishing the colours red, blue and green.
7. Failure to adhere to the outlined protocol and reagents may affect the performance and lead to false positive/negative results.
8. The probe should not be diluted or mixed with other probes.
9. 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.

Storage and Handling

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 probe remains stable throughout the freeze-thaw cycles experienced during normal use (where one cycle constitutes the probe's removal from and replacement into the freezer) and is photostable for up to 48 hours after being exposed to continuous lighting conditions. 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]
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 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.

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 autofluorescence. 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 making¹⁰.

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:** slides should be spotted 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.**)

Stability of Finished Slides

Finished 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 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 prior to using the test for diagnostic purposes.
8. Suboptimal conditions may result in non-specific binding that may be misinterpreted as a probe signal.

Interpretation of Results

Assessing Slide Quality

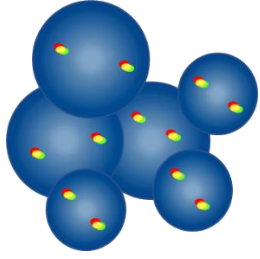
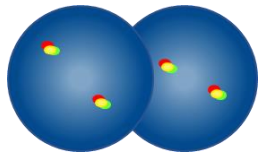
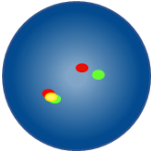
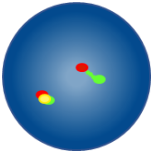
The slide should not be analysed if:

- Signals are too weak to analyse in single filters - in order to proceed with analysis, signals should appear bright, distinct and easily evaluable
- There are high numbers of clumped/overlapping cells obstructing the analysis
- >50% of the cells are not hybridised
- There is excess of fluorescent particles between cells and/or a fluorescent haze that interferes with the signals - in optimal slides the background should appear dark or black and clean
- Cell nucleus borders cannot be distinguished and are not intact

Analysis Guidelines

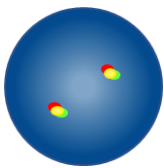
- Two analysts should analyse and interpret each sample. Any discrepancies should be resolved by assessment by a third analyst
- Each analyst should be suitably qualified according to recognised national standards
- Each analyst should score independently 100 nuclei for each sample. The first analyst should start the analysis from the left side of the slide and the second analyst from the right one

- Each analyst should document their results in separate sheets
- Analyse only intact nuclei, not overlapped or crowded nuclei or nuclei covered by cytoplasmic debris or high degree of autofluorescence
- Avoid areas where there is excess of cytoplasmic debris or non-specific hybridisation
- Signal intensity may vary, even with a single nucleus. In such cases, use single filters and/or adjust the focal plane
- In suboptimal conditions signals may appear diffuse. If two signals of the same colour touch each other, or the distance between them is no greater than two signal widths, or when there is a faint strand connecting the two signals, count as one signal
- When analysing dual-colour breakapart probes, if there is a gap between the red and green signal no greater than two signal widths apart, count as not rearranged/fused signal
- If in doubt about whether a cell is analysable or not, then do not analyse it

Analysis Guidelines	
	Do not count – nuclei are too close together to determine boundaries
	Do not count overlapping nuclei – all areas of both nuclei are not visible
	Count as two fusion signals - the gap between the red and green signal is less than two signal widths
	Count as two fusion signals - one fusion signal is diffuse

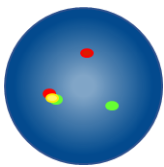
Expected Results

Expected normal signal pattern



In a normal cell, two red/green fusion signals (2F) are expected.

Expected abnormal signal pattern



In a cell with a balanced *AML1 (RUNX1)* rearrangement, the expected signal pattern will be one red, one green and one fusion signal (1R, 1G, 1F).

Other signal patterns are possible in aneuploid/unbalanced specimens.

Known Cross-Reactivity

No known cross-reactivity.

Adverse Event Reporting

If you believe this device has malfunctioned or suffered a deterioration in its performance characteristics which may have contributed to an adverse event (e.g. delayed or misdiagnosis, delayed or inappropriate treatment), this must be reported immediately to the manufacturer (email: vigilance@ogt.com).

If applicable, the event should also be reported to your national competent authority. A list of vigilance contact points can be found at: <http://ec.europa.eu/growth/sectors/medical-devices/contacts/>.

Specific Performance Characteristics

Analytical Specificity

Analytical specificity is the percentage of signals that hybridise to the correct locus and no other location. The analytical specificity was established by analysing a total of 200 target loci. The analytical specificity was calculated as the number of FISH signals that hybridised to the correct locus divided by the total number of FISH signals hybridised.

Table 1. Analytical Specificity for the AML1 Breakapart Probe

Probe	Target Locus	No. of Signals Hybridised to the Correct Locus	Total No. of Signals Hybridised	Specificity (%)
Red AML1	21q22	200	200	100
Green AML1	21q22	200	200	100

Analytical Sensitivity

Analytical sensitivity is the percentage of scoreable interphase cells with the expected normal signal pattern. The analytical sensitivity was established by analysing interphase cells across different normal samples. The sensitivity was calculated as the percentage of scoreable cells with the expected signal pattern (with a 95% confidence interval).

Table 2. Analytical Sensitivity for the AML1 Breakapart Probe

No. of Cells with Expected Signal Patterns	No. of Cells with Scoreable Signals	Sensitivity (%)	95% Confidence Interval
4974	5000	99.48	99.24 – 99.64

Characterisation of Normal Cut-off Values

The normal cut-off value, in association with FISH probes, is the maximum percentage of scoreable interphase cells with a specific abnormal signal pattern at which a sample is considered normal for that signal pattern.

The normal cut-off value was established using samples negative for the rearrangement that the probe is intended to detect and the beta inverse function. For each sample, the signal patterns of 100 interphase nuclei were recorded by two independent analysts, totalling 200 per sample.

Table 3. Characterisation of Normal Cut-off Values for the AML1 Breakapart Probe

Abnormal signal pattern	Number of samples analysed to generate cut-off	Number of nuclei evaluated per sample	Max. no. of false positive signal patterns	Normal cut-off value (%)
1R, 1G, 1F	25	200	2	3.5

Laboratories must verify cut-off values using their own data^{11, 12}.

Precision and Reproducibility

Reproducibility was established by three individual laboratories which tested six blinded samples (two negative for the rearrangement, two low positive samples which were 1 to 3 times the cut-off and two high positive samples which contained more than 45% of cells positive for the rearrangement). The analysis was conducted using two replicates of each sample over the course of five non-consecutive days.

All three sites carried out intra-day, inter-day and inter-site testing using the same lot of probe, whilst one of the sites also carried out inter-lot reproducibility using three different lots of probe.

The reproducibility was calculated using the agreement between the variables examined during each test.

Table 4. Reproducibility and Precision for the AML1 Breakapart Probe

Reproducibility study	Sample	Agreement (%)
Intra-day / inter-day / inter-site	Negative	100
	High Positive	100
Inter-lot	Negative	100
	High Positive	100

Clinical Performance

The clinical performance was established using a representative set of 100 consecutive samples taken from patients referred for AML or MDS. The incident rates of the rearrangements detected by the probe were compared with those gathered from a review of literature sources.

To enable this comparison, the confidence interval indicated by the literature in a population size of 100 samples was calculated by computing 1 – sample proportions test with continuity correction.

Table 5. Clinical Performance for the AML1 Breakpart Probe

Rearrangement	Prevalence			
	Literature Review (%)	95% LCI (%)	Clinical Study (%)	95% UCL (%)
AML with RUNX1 rearrangements	3.8	1.2	2	10.2

Additional Information

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

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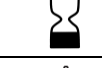
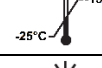


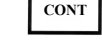
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W: www.ogt.com

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Guide to Symbols

	en: Catalogue number
	en: <i>In vitro</i> diagnostic medical device
	en: Batch code
	en: Consult instructions for use
	en: Manufacturer
	en: Use-by date
	en: Temperature limit
	en: Keep away from sunlight
	en: Contains sufficient for <n> tests
	en: Contents

Patents and Trademarks

CytoCell is a registered trademark of CytoCell Ltd.



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