A Sysmex Group Company

CytoCell

Package Insert

REF: CDA-LPH013

Name: KMT2A Breakapart FISH Probe Kit PDx

IVD Rx Only

ogt.com/IFU

Further information available at ogt.com/IFU

Indications for Use

The CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx is a fluorescence *in situ* hybridization (FISH) test used to detect rearrangement of the *KMT2A* region on chromosome 11 at location 11q23.3 in 3:1 methanol/glacial acetic acid fixed bone marrow specimens from patients with acute leukemia with *KMT2A* rearrangement.

The assay is indicated for detecting the presence of rearrangements involving the *KMT2A* region as a companion diagnostic to aid in identifying those patients for whom treatment with REVUFORJ® (revumenib) is indicated in accordance with the approved therapeutic product labeling. The CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx is not intended for monitoring of residual disease.

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 *KMT2A* gene. Breakpoints outside of this region, or variant rearrangements wholly contained within this region, may not be detected with this device.
This device is not intended for monitoring of residual disease or for use as prenatal test, or population-based screening test.
This device has not been validated for sample types, disease types, or purposes outside of those stated in the indications for use.
Reporting and interpretation of FISH results should be performed by a qualified pathologist or cytogeneticist.
Reporting and interpretation of FISH results should be consistent with professional standards of practice and should take into consideration other clinical and diagnostic information. Failure to adhere to the protocol may affect the performance and lead to false results.
The reproducibility of the test in samples with positivity ranging between 3.1-6.2% for ALL and 3.8-7.6% for AML are 65.3% and 75%, respectively. Therefore, use caution when interpreting results within this range.
Analytical validation demonstrating performance is limited to 1F1R1G signal pattern. Other signal patterns that are considered positive for *KMT2A* rearrangement have not been validated.
This device is intended for in vitro diagnostic use only.
For prescription use only.

Summary and Explanation of the Test

The CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx targets the *KMT2A* gene, with a probe design which flanks the *KMT2A* breakpoint region. This design detects the presence of a rearrangement involving the *KMT2A* gene, irrespective of the rearrangement partner; a positive breakapart signal pattern with CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx indicates the presence of a *KMT2A* rearrangement.

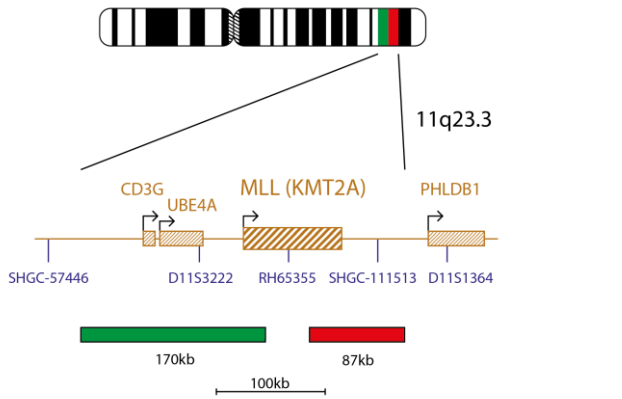
The *KMT2A* (lysine methyltransferase 2A) gene at 11q23.3 is commonly rearranged in acute leukemias, especially in infant leukemia. *KMT2A* rearrangements can be detected in approximately 70%-80% of infants with acute lymphoblastic leukemia (ALL) and in 5-10% of pediatric and adult ALLs[1,2]. They can also be found in more than 50% of infants with acute myeloid leukemia (AML) and is also seen in 10% of adolescents and 2-3% of adults with AML. *KMT2A* rearrangements are also seen in mixed-phenotype acute leukemia, a rare type of acute leukemia more common in infants and children than adults[2]. To date, more than 90 partners have been identified with the most common partner genes being *AFF1* (4q21), *MLLT3* (9p22) and *MLLT1* (19p13.3)[2].

The *KMT2A* gene encodes for a transcription factor which plays an important role in embryonic development and hematopoiesis as an important epigenetic regulator. The *KMT2A* rearrangements lead to the expression of *KMT2A* fusion proteins that interact with the nuclear protein menin. This *KMT2A* fusion protein – menin interaction leads to aberrant expression of *HOX* genes and *MEIS1*, causing a hematopoietic transformation block and leukemic transformation[3,4,5].

Probe Specification

KMT2A, 11q23.3, Red
KMT2A, 11q23.3, Green

CMP-H036 v006.00



The *KMT2A* product consists of an 87kb probe, labelled in red, covering a region telomeric to the *KMT2A* gene including the marker SHGC-111513 and a green probe covering a 170kb region centromeric to the *KMT2A* gene spanning the *CD3G* and *UBE4A* genes.

Principles of the Procedure

Fluorescence *in situ* hybridization (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 hybridize to entire chromosomes or single unique sequences. Target DNA, after fixation and denaturation, is available for annealing to a similarly denatured, fluorescently labelled DNA probe, which has a complementary sequence. Following hybridization, unbound and non-specifically bound DNA probe is removed, and the DNA is counterstained for visualization. Fluorescence microscopy then allows the visualization of the hybridized probe on the target material.

Materials Provided

Probe: 100µl per vial (10 tests)
The probes are provided premixed in hybridization solution (<65% formamide; <20mg dextran sulfate; <10% of 20x 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 In Vitro Diagnostic Use.
2. Caution: Federal law restricts this device to sale by or on the order of a physician. (Rx 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. Handle DAPI with care; wear gloves and a lab coat.
5. Do not use if the vial(s) are damaged, or the vial contents are compromised in any way.
6. 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.
7. 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.
8. Operators must be capable of distinguishing the colors red, blue and green.
9. Failure to adhere to the outlined protocol and reagents may affect the performance and lead to false positive/negative results.
10. Handle all biological specimens using standard laboratory precautions, as they should be considered potentially infectious regardless of their known or presumed status.

Labeling according to OSHA Hazard Communication Standard

Label elements – FISH Probe

Contains Formamide <70%
Contains Dextran sulfate sodium <20%

Danger

Hazard Statements
Causes skin irritation.
Causes serious eye irritation.
May damage the unborn child.

Precautionary Statements - Prevention
Obtain special instructions before use.
Do not handle until all safety precautions have been read and understood.
Wear protective gloves/clothing and eye/face protection.
Wash face, hands and any exposed skin thoroughly after handling.

Precautionary Statements - Response
IF exposed or concerned: Get medical advice/attention.
Specific treatment (see supplemental first aid instructions on this label).
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
If eye irritation persists: Get medical advice/attention.
IF ON SKIN: Wash with plenty of water and soap.
If skin irritation occurs: Get medical advice/attention.
Take off contaminated clothing and wash it before reuse.

Precautionary Statements - Storage
Store locked up.

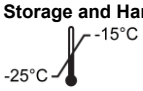
Precautionary Statements - Disposal
Dispose of contents/container to an approved waste disposal plant.

Refer to the Safety Data Sheet for more information.

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
- 4°C / Cold / Refrigerator: +2°C to +8°C

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.

The FISH probe, DAPI Antifade ES counterstain, and Hybridization 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) 10 cycles for the 100µl (10 tests) vial of FISH probe, and 15 cycles for the 150µl (15 tests) vial of counterstain. Exposure to light should be minimized 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.

All biological specimens should be treated as potentially infectious regardless of their known or presumed status. Handle all biological specimens using standard laboratory precautions.

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. 15mL conical centrifuge tube
6. Fluorescence microscope (Please see Fluorescence Microscope Recommendation section)
7. Phase contrast microscope
8. Clean plastic, ceramic or heat-resistant glass Coplin jars
9. Forceps
10. Calibrated pH meter (or pH indicator strips capable of measuring pH 6.5 – 8.0)
11. Humidified, lightproof container
12. Fluorescence grade microscope lens immersion oil
13. Bench top centrifuge
14. Microscope slides
15. 24x24mm coverslips
16. Timer
17. 37ºC incubator
18. Rubber solution glue
19. Vortex mixer
20. Graduated cylinders
21. Magnetic stirrer
22. Calibrated thermometer
23. Clear nail varnish
24. Freezer

Optional Equipment not Supplied

1. Cytogenetic drying chamber
2. Fume hood

Reagents Needed but not Supplied

1. Complete culture medium
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
8. 100% Methanol
9. 100% Acetic Acid (Glacial)
10. 0.075M Hypotonic Potassium Chlorine solution (KCl) warmed to 37°C

Sample Collection and Transport
Bone marrow sample collections should be performed according to the laboratory institution guidelines. The AGT *Cytogenetics Laboratory Manual*[6] contains recommendations for bone marrow sample collection, culturing, harvesting and slide making. Bone marrow samples should be collected and transported in heparin tubes and shipped at ambient (15°C to 25°C) temperature. Samples must not be frozen or stored on ice. Bone marrow samples should be processed immediately or within 72 hours post-aspiration and subsequently stored cold at 4°C (2°C to 8°C). Bone marrow samples should be cultured at a concentration of $1x10^6$ cells per mL before processing. See Sample Processing section below.

Sample Processing
Note: Fixative (3:1 methanol/acetic acid) should be prepared fresh daily. Fixative should be kept in the freezer (-25°C to -15°C) wherever possible.

1. Bone marrow samples should be cultured at a concentration of $1x10^6$ cells per mL in complete culture medium in a 15mL conical centrifuge tube (total volume should not exceed 10mL).
2. Centrifuge the tube at 220 x g for 10 minutes.
3. Remove as much supernatant as possible without disturbing the pellet. Resuspend the pellet by gently agitating the tube.
4. Add 9mL of pre-warmed (37°C) KCl to the tube and mix by inverting the tube gently several times.
5. Incubate tube at 37°C for 28 minutes.
6. Add ~0.5mL of fixative to the cell suspension and mix by inverting the tube gently several times.
7. Centrifuge the tube at 220 x g for 8 minutes.
8. Remove as much supernatant as possible without disturbing the pellet. Resuspend the pellet by gently agitating the tube.
9. Using a vortex mixer set at three quarters speed to mix the pellet, slowly add fixative solution drop-by-drop to the tube. Continue to add fixative until the color is dark-brown and no foamy bubbles appear at the top of the solution. At this point top the tube up to 10mL with fixative solution and mix by inverting the tube several times.
10. Centrifuge the tube at 220 x g for 8 minutes.
11. Remove as much supernatant as possible without disturbing the pellet. Resuspend the pellet by gently agitating the tube.
12. Add 6mL of fixative to the tube and resuspend the pellet by inverting the tube several times.
13. Centrifuge the tube at 220 x g for 8 minutes.
14. Repeat the fixative wash steps (steps 11 to 13) two more times or until the fixative is clear.
15. Add 4mL of fixative to the tube and resuspend the pellet by inverting the tube several times.
16. Proceed to Slide Preparation (Step 17) or alternatively fixed pellets can be stored at -20°C.

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 4 weeks 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 DAPI counterstain to laboratory lights is limited at all times).

Slide Preparation
17. Centrifuge the tube at 220 x g for 8 minutes.
18. Resuspend the pellet in fixative, adjusting the volume of the cell suspension to achieve an appropriate density for analysis.
19. Spot the cell sample onto an appropriately labeled glass 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).
20. Assess the cell density using a phase contrast microscope. If the number of cells in a 10x field of view is <50, repeat centrifugation and re-suspend the pellet in a lower volume of fixative.
21. Immerse the slide in 2xSSC for 2 minutes at room temperature (RT) without agitation.
22. Dehydrate in an ethanol series (70%, 85% and 100%), each for 2 minutes at RT.
23. Allow to dry.

Pre-Denaturation
24. Remove the probe from the freezer and allow it to warm to RT. Briefly centrifuge tubes before use.
25. Ensure that the probe solution is uniformly mixed with a pipette.
26. Remove 10µl of probe per test, and transfer it to a microcentrifuge tube. Quickly return the remaining probe to the freezer.
27. Place the probe and the sample slide to prewarm on a 37ºC (+/- 1ºC) hotplate for 5 minutes.
28. 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
29. Denature the sample and probe simultaneously by heating the slide on a hotplate at 75ºC (+/- 1ºC) for 2 minutes.

Hybridization
30. Place the slide in a humid, lightproof container at 37ºC (+/- 1ºC) overnight (16 to 18 h).

Post-Hybridization Washes
31. Remove the DAPI counterstain vial from the freezer and allow it to warm to RT.
32. Remove the coverslip and all traces of glue carefully.
33. Immerse the slide in 0.4xSSC (pH 7.0) at 72ºC (+/- 1ºC) for 2 minutes without agitation.
34. Drain the slide and immerse it in 2xSSC, 0.05% Tween-20 at RT (pH 7.0) for 30 seconds without agitation.
35. Drain the slide and apply 10µl of DAPI antifade onto each sample.
36. Put the DAPI counterstain vial back into the freezer.
37. Cover with a coverslip, remove any bubbles, edge with clear nail varnish and allow the color to develop in the dark for 10 minutes.
38. View with a fluorescence microscope (see **Fluorescence Microscope Recommendation**).

Stability of Finished Slides
Hybridized slides remain analyzable for up to 4 weeks if stored in the dark at refrigerator temperature.

Fluorescence Microscope Recommendation
Use a 100-watt mercury lamp or equivalent and oil immersion plan apochromat objectives 60/63x or 100x for optimal visualization. The fluorophores used in this probe set will excite and emit at the following wavelengths:

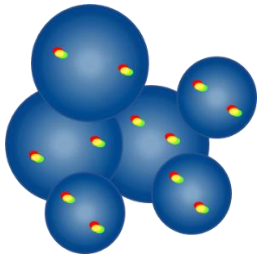
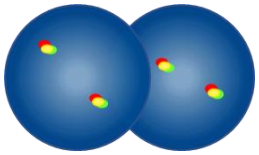
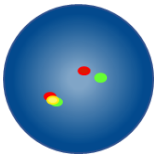
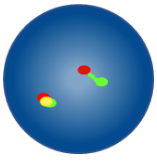
Fluorophore	$Excitation_{max}$ [nm]	$Emission_{max}$ [nm]
Green	495	521
Red	596	615

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 visualization 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 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.

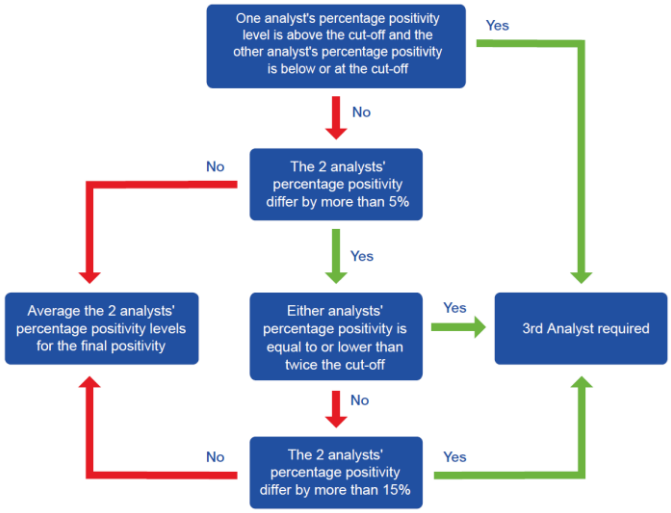
Interpretation of Results
Assessing Slide Quality
The slide should not be analyzed if:
- Signals are too weak to analyze in single filters - in order to proceed with analysis, signals should appear bright, distinct and easily evaluable
- There are high numbers of clumped/overlapping cells obstructing the analysis
- >50% of the cells are not hybridized
- 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 analyze and interpret each sample. Any discrepancies should be resolved by assessment by a third analyst
- Each analyst should be suitably qualified according to recognized 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
- Analyze 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 hybridization
- 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 color 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 analyzing dual-color breakapart probes, if there is a gap between the red and green signal no greater than 2 signals width apart, count as not rearranged/fused signal
- If in doubt about whether a cell is analyzable or not, then do not analyze it
- Refer to associated interpretation guide (OGT.com/IFU) for further information and representative images.

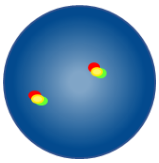
Analysis Guidelines	
	Do not count – nuclei are too close together to determine boundaries
	Do not count overlapping nuclei – all areas of both nuclei are not visible
	Count as two fusion signals - the gap between the red and green signal is less than two signal widths
	Count as two fusion signals -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

Third analyst requirements
Depending on the number of abnormal nuclei each analyst has seen, a third analyst may be required. The third analyst will also analyze 100 nuclei. The rules for a third analyst are detailed in the text and flowchart below.
A third analyst is required if:
- One of the analysts' percentage positivity is above the cut-off and the other analysts' percentage positivity is below or at the cut-off.
- The two analysts' percentage positivity differ by more than 5% and either analyst's percentage positivity is equal to or lower than twice the cut-off.
- The two analysts' percentage positivity differ by more than 15%.

Of the three scores obtained, the two closest positive per values will be averaged for the final positivity. If the 3 scores are equidistant then the median positivity value should be used.

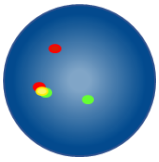


Expected Normal Signal Pattern



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

Expected Abnormal Signal Pattern



In a cell with a balanced *KMT2A* rearrangement, the expected signal pattern will be one red/green fusion, one red and one green (1F1R1G).

KMT2A Abnormal Signal Patterns
Other signal patterns are possible in samples with aneuploid/unbalanced rearrangements. A signal pattern is considered *KMT2A* abnormal (indicative of *KMT2A* rearrangement) when it includes:

nFnRnG, where:
- **nF** (number of Fusion signals) ≥ 0
- **nR** (number of Red signals) ≥ 0
- **nG** (number of Green signals) ≥ 1

Any signal pattern fitting this description may be interpreted as consistent with *KMT2A* rearrangement and revumenib eligibility.

Procedural Recommendations
1. Baking or ageing of slides may reduce signal fluorescence.
2. Hybridization 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, water baths 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 hybridization can result in additional or unexpected signals.
7. Users should optimize the protocol for their own samples prior to using the test.
8. Suboptimal conditions may result in non-specific binding that may be misinterpreted as a probe signal.

Known Relevant Interferences / Interfering Substances
No known relevant interferences / interfering substances.

Known Cross-Reactivity
No known cross-reactivity.

Specific Performance Characteristics

1. Analytical Specificity
Analytical specificity is the percentage of signals that hybridize to the correct locus and no other location. The analytical specificity of the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx was established by analyzing a total of 200 target loci from metaphase chromosomes prepared from five normal male peripheral blood samples. The analytical specificity was calculated as the number of FISH signals that hybridized to the correct locus divided by the total number of FISH signals hybridized. The result was then multiplied by 100, to give a percentage, and is given with a 95% confidence interval. The analytical specificity of the KMT2A Breakapart FISH Probe Kit PDx was 100%, as shown in Table 1.

Table 1: Analytical Specificity for the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx

Probe	Target	Number of metaphase chromosomes hybridized	Number of correctly hybridized loci	Analytical Specificity (%)	95% Confidence Interval (%)
KMT2A, Red	11q23.3	200	200	100	98.12 - 100
KMT2A, Green	11q23.3	200	200	100	98.12 - 100

2. Analytical Sensitivity
Analytical sensitivity is the percentage of scoreable interphase cells that exhibit the expected normal signal pattern. The sensitivity of the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx was evaluated using interphase nuclei from karyotypically normal bone marrow samples, representative of the intended population.
Each sample was analyzed by two independent analysts, with each analyst scoring 100 nuclei per sample (200 nuclei per sample in total), resulting in 5,000 scorable nuclei evaluated across 25 samples for each study. The sensitivity was calculated as the percentage of cells with the expected signal pattern, and the result is reported with a 95% confidence interval.

Table 2: Analytical Sensitivity for the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx

Study	No. of cells with expected signal patterns	Total number of cells with scoreable signals	Analytical Sensitivity (%)	95% Confidence interval (%)
Study 1	4965	5000	99.30	99.04, 99.54
Study 2	4955	5000	99.10	98.81, 99.35

3. Characterization of Normal Cut-off Values
The limit of detection (LoD) for a FISH test is determined by calculating the upper limit of the abnormal signal pattern in normal cells, known as the "normal cut-off value." This value represents the maximum percentage of scoreable interphase cells exhibiting a specific abnormal signal pattern, above which a sample is considered positive for the detected rearrangement.
An analytical result above the normal cut-off (upper reference limit) indicates the presence of the rearrangement, while results below the cut-off are considered negative.
The normal cut-off for the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx was established using data from 1,600 AML and 25 ALL bone marrow samples negative for the rearrangement. Two independent analysts each analyzed 100 interphase nuclei per sample.
For the '1F1R1G' signal pattern, the normal cut-off value was calculated to be 3.8% for AML and 3.1% for ALL. Analytical validation demonstrating performance is limited to 1F1R1G signal pattern. Other signal patterns that are considered positive for *KMT2A* rearrangement have not been validated.

Table 3: Characterization of Normal Cut-off Values for the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx

Disease	Abnormal signal pattern	No. samples analyzed to generate cut-off	No. nuclei evaluated per sample	Max. no false positive signal patterns	Normal cut-off value (%)
AML	1F1R1G	1600	200	3	3.8
ALL	1F1R1G	25	200	2	3.1

The product is for professional use only and is intended to be interpreted by a qualified Pathologist or Cytogeneticist.[7,8]

4. Reproducibility

Reproducibility of the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx has been assessed across multiple factors to evaluate variability in results. Studies were conducted to examine site-to-site, day-to-day, operator-to-operator and lot-to-lot variability using bone marrow samples fixed in 3:1 methanol/acetic acid from patients with AML and ALL. Reproducibility metrics were met, confirming the kit's reliability across different testing conditions.

All studies conducted tested Negative samples, Near Cut-Off/Low Positive samples and High Positive samples from both AML and ALL patients. Negative samples achieved >99% agreement with predicted Negative class across all studies in all tested conditions. High Positive samples achieved 100% agreement with predicted Positive class across all studies in all tested conditions. Near Cut-Off/Low Positive samples achieved 63-100% agreement with predicted Near Cut-Off/Low Positive class across all studies in all tested conditions.

All replicates in all studies were analyzed by two analysts with a target of a total of 200 cells per replicate and both analysts recorded the signal patterns seen in each replicate.

Acute Myeloid Leukemia (AML) fixed bone marrow samples:

Site-to-site and day-to-day reproducibility for AML samples were evaluated by analyzing two replicates of six samples (two High Positive, two Near Cut-Off/Low Positive, and two Negative) across five days at three sites.
Lot-to-lot reproducibility was assessed at a single site by analyzing four replicates of three samples (one High Positive, one Low Positive and one Negative) across three different probe lots.
Analysis showed 100% agreement for both the High Positive and Negative samples, demonstrating consistent performance across sites, days and probe lots.

Table 4: Specimen Agreement Site-to-Site Reproducibility for the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx using fixed AML samples

Disease	Sample	Agree	Disagree	Total	Agreement (%)
AML	**Negative 1**	30	0	30	100
AML	**Negative 1**	30	0	30	100
AML	**Near cut-off 1**	19	11	30	63
AML	**Near cut-off 2**	27	3	30	90
AML	**Positive 1**	30	0	30	100
AML	**Positive 2**	30	0	30	100

Table 5: Site-to-Site Analysis of Variance for the KMT2A Breakapart FISH Probe Kit PDx using fixed AML samples

Disease	Sample	N	Mean of abnormal percentage	Intra-Day SD	Inter-Day SD	Inter-site SD	Total SD
AML	**Negative 1**	30	0.00	0.00	0.00	0.00	0.00
AML	**Negative 2**	30	0.02	0.00	0.00	0.00	0.09
AML	**Near cut-off 1**	30	4.12	0.00	0.00	1.17	1.98
AML	**Near cut-off 2**	30	7.35	0.00	1.69	0.87	3.12
AML	**Positive 1**	30	76.15	0.00	0.77	4.57	6.60
AML	**Positive 2**	30	84.85	0.81	0.35	3.06	4.14

Table 6: AML Lot-to-Lot Study Results Summary

Disease	Sample	Agree	Disagree	Total	Agreement
AML	**Negative (N2)**	12	0	12	100.0%
AML	**Near Cut-Off (NCO2)**	12	0	12	100.0%
AML	**High Positive (HP2)**	12	0	12	100.0%

Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL) fixed bone marrow samples

Reproducibility, which measures the variability of test results, was assessed in a retrospective 3-site study using 3:1 methanol/acetic acid-fixed bone marrow samples from AML and ALL patients. This study evaluated intra-day, inter-day, inter-site, and inter-operator reproducibility to establish the test's precision. Twelve fixed bone marrow specimens were tested across different *KMT2A* positivity levels: two Negative (N), two Near Cut-Off (NCO) and two High Positive (HP) samples, for both AML and ALL. Testing was conducted with two replicates of each sample per day, using two probe lots, by two operators at each site, over three non-consecutive days across three sites. The study met predefined acceptance criteria. Negative Samples: ≥90% agreement with the predicted negative classification. High Positive Samples: ≥95% agreement with the predicted positive classification.

Table 7: 3-Site Reproducibility Study Results Summary for the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx using Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL) fixed bone marrow samples

Disease	Sample	Agree	Disagree	Total	Agreement
ALL	**Negative (2)**	144	0	144	100.0%
ALL	**Near Cut-Off (2)**	94	50	144	65.3%
ALL	**High Positive (2)**	144	0	144	100.0%
AML	**Negative (2)**	143	1	144	99.3%
AML	**Near Cut-Off (2)**	108	36	144	75.0%
AML	**High Positive (2)**	144	0	144	100.0%

Table 8: Site-to-Site Analysis of Variance for the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx using Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL) fixed bone marrow samples

Disease	Sample	N	Mean of abnormal percentage	Intra-Day SD	Inter-Day SD	Intra-site SD	Inter-Operator SD	Total SD
ALL	**Negative 1**	72	0.15	0.32	0.00	0.17	0.00	0.36
ALL	**Negative 2**	72	0.27	0.34	0.16	0.17	0.00	0.41
ALL	**Near cut-off 1**	72	3.36	1.65	0.00	1.03	1.16	2.27
ALL	**Near cut-off 2**	72	5.01	2.29	0.49	0.00	0.00	2.34
ALL	**Positive 1**	72	58.20	5.63	2.41	1.15	0.00	6.24
ALL	**Positive 2**	72	73.49	3.38	1.44	0.77	1.00	3.88
AML	**Negative 1**	72	0.28	0.42	0.14	0.00	0.00	0.45
AML	**Negative 2**	72	0.39	0.51	0.13	0.13	0.36	0.67
AML	**Near cut-off 1**	72	4.79	2.49	0.00	0.35	0.00	2.53
AML	**Near cut-off 2**	72	5.41	1.89	0.00	0.16	0.96	2.13
AML	**Positive 1**	72	50.12	9.96	0.00	0.00	5.24	11.49
AML	**Positive 2**	72	76.33	5.27	0.00	3.53	0.00	6.61

Acute Lymphoblastic Leukemia (ALL) fixed bone marrow samples

Lot-to-lot reproducibility was assessed using 3:1 methanol/acetic acid-fixed bone marrow samples from patients with ALL across varying *KMT2A* positivity levels: one Negative (N), one Near Cut-Off (NCO) and one High Positive (HP) sample. Each sample was tested in four replicates per day, using three different probe lots, by one operator, in a single day at one site. The results demonstrate consistent performance across different reagent lots, with high agreement percentages for all tested positivity levels.

Table 9: ALL Lot-to-Lot Study Results Summary for the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx

Disease	Sample	Agree	Disagree	Total	Agreement
ALL	**Negative (N1)**	12	0	12	100.0%
ALL	**Near Cut-Off (NCO2)**	11	1	12	91.7%
ALL	**High Positive (HP1)**	12	0	12	100.0%

5. Clinical Performance

A single site retrospective bridging study using blinded, randomized de-identified 3:1 methanol/acetic acid-fixed bone marrow samples was conducted to determine the clinical performance of the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx as a companion diagnostic assay (CDx).

Revumenib is an oral menin inhibitor, for the treatment of relapsed or refractory acute leukemia with a lysine methyltransferase 2A gene (*KMT2A*) rearrangement in adult and pediatric patients 1 year and older. A Phase 1/2, Open label, Dose escalation and Dose-Expansion Cohort study trial for the SNDX-5613 intervention revumenib (SNDS-5613-0700) was performed by Syndax Pharmaceutical Inc.; patient samples from this trial and procured samples were utilized in assessing clinical performance of the CDx.

Clinical performance was determined by clinical bridging of the CDx result to a Clinical Trial Assay (CTA) result which was used to enroll patients into the Phase 1/2 SNDX-5613-0700 trial, and through calculation of primary efficacy defined by the rate of complete remission (CR) or CR with partial hematologic recovery (CRh) in the CDx positive population.

Of the 139 *KMT2A* rearrangement positive patients identified by local diagnostic testing and enrolled into the trial, 63 patient samples were available for retrospective testing with the CDx. An additional 102 local diagnostic test *KMT2A* rearrangement negative samples were obtained and tested with the CDx. These samples included *KMT2A* rearrangement negative SNDX-5613-0700 trial patients (n=12) and procured samples (n=90). The negative samples in combination with the 63 positives available for CDx testing form the CDx-evaluable cohort.

The acceptance criteria for Clinical Bridging study were ≥95% concordant classification (for positive, negative and overall agreement) for the aberration being detected between the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx and the local diagnostic testing result. Concordance between the CDx and the local diagnostic test result is detailed in table 10. Acceptance criteria for all agreements were met.

Table 10 - Concordance between CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx and Clinical Trial Assay (CTA).

	CDx (CTA as reference)	
Measure of agreement	**Percent agreement**	**95% CI** *(Exact Clopper-Pearson)*
PPA	95.2% (60/63)	86.7%, 99.0%
NPA	100.0% (102/102)	96.4%, 100.0%
OPA	98.2% (162/165)	94.8%, 99.6%

Data includes all CDx-evaluable patients in Phase 1/2 of SNDX-5613-0700.
PPA = Positive percent agreement, NPA = Negative percent agreement, OPA = Overall percent agreement.

Efficacy was estimated from the CDx-evaluable population for patients enrolled into the SNDX-5613-0700 trial and tested positive using the CDx, as the proportion achieving complete remission (CR) or CR with partial hematologic recovery (CRh). Response rate is detailed in table 11 below.

Table 11 - CR/CRh for CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx positive patients.

Best Response	**Count (N=60)**	**Percentage (95% CI)** *(exact Clopper-Pearson)*
CR/CRh	15	25.0% [14.7%, 37.9%]
CR – Complete Remission	10	16.7% [8.3%, 28.5%]
CRh – CR with partial hematologic recovery	5	8.3% [2.8%, 18.4%]
Other response*	45	75.0% [62.1%, 85.3%]

Includes best response data for all CDx-evaluable patients in Phase 1/2 of SNDX-5613-0700.
Other responses to therapy include: CRi – CR with incomplete hematologic recovery, CRp – CR with incomplete platelet recovery, MLFS – Morphologic leukemia free state, No response, PD – Progressive disease, PR – Partial remission, Response missing, and Unknown.

The results of the clinical bridging study identified that primary efficacy in CDx-evaluable patients treated with revumenib was 25.0% [14.7%, 37.9%] and confirmed that the CDA-LPH013 KMT2A Breakapart FISH Probe Kit PDx successfully met all predefined acceptance criteria, demonstrating its reliability and effectiveness in detecting *KMT2A* rearrangements in relapsed or refractory acute leukemia cases.

Additional Information

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

ISO 15223-1:2021 - "Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements"
(© International Organization for Standardization)

Symbol	Title	Description	Reference Number(s)
	**en:** Manufacturer	Indicates the medical device manufacturer	5.1.1
	**en:** Use-by date	Indicates the date after which the medical device is not to be used	5.1.4
	**en**: Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified	5.1.5
	**en:** Catalogue number	Indicates the manufacturer's catalogue number so that the medical device can be identified	5.1.6
	**en:** Keep away from sunlight	Indicates a medical device that needs protection from light sources	5.3.2
	**en:** Temperature limit	Indicates the temperature limits to which the medical device can be safely exposed	5.3.7
	**en**: Biological risks	Indicates that there are potential biological risks associated with the medical device	5.4.1
 ogt.com/IFU	**en:** Consult electronic instructions for use	Indicates the need for the user to consult the instructions for use	5.4.3
	**en:** Caution	Indicates that caution is necessary when operating the device or control close to where the symbol is placed, or that the current situation needs operator awareness or operator action in order to avoid undesirable consequences	5.4.4
	**en:** *In vitro* diagnostic medical device	Indicates a medical device that is intended to be used as an in vitro diagnostic medical device	5.5.1
	**en:** Contains sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device	5.5.5
	**en:** Unique Device Identifier	Indicates a carrier that contains unique device identifier information	5.7.10

U.S. FDA Final Rule "Use of Symbols in Labeling", 81 FR 38911, June 2016

Symbol	Title	Description	Reference Number(s)
	**en:** For Prescription Use Only	Caution: Federal law restricts this device to sale by or on the order of a physician	21 CFR § 801.15, § 801.109, § 809.10

EDMA symbols for IVD reagents and components, October 2009 revision

Symbol	Title	Description	Reference Number(s)
	**en:** Contents (or contains)	N/A	N/A

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Package Insert Version History

Version	Date of approval	Change
V001	19-09-2025	Initial version