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Product Catalogue Number FP026 **For Research Use Only – RUO**

KMT2A (MLL) Gene Breakapart Probe Detection Kit

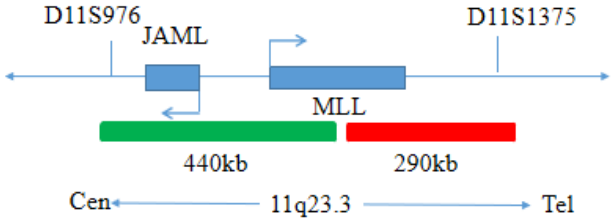
[Product Name] KMT2A (MLL) Gene Breakapart Probe Detection Kit (Fluorescence In-Situ Hybridization Method).

[Product Introduction]
The kit uses orange fluorescein-labeled MLL probe and green fluorescein-labeled MLL probe to bind the MLL probe to the target detection site by in situ hybridization.

[Product Composition]
The kit consists of MLL dual-color probes, as shown in Table 1.

Table 1 Kit composition

Package Specifications	Component name	Specifications	Quantity	Main components
10 Tests/box	MLL dual color probe	100μL/Tube	1	MLL Orange probe, MLL Green probe



[Storage conditions & Validity]
Keep sealed away from light at -20°C±5°C. The product is valid for 12 months. Avoid unnecessary repeated freezing and thawing that should not exceed 10 times. After opening, within 24 hours for short-term preservation, keep sealed at 2-8°C in dark. For long-term preservation after opening, keep the lid sealed at -20°C±5°C away from light. The kit is transported below 0°C.

[Applicable Instruments]
Fluorescence microscopy imaging systems, including fluorescence microscopy and filter sets suitable for DAPI (367/452), Green (495/517), and Orange (547/565).

[Sample Requirements]
1. Applicable specimen type: unfixed fresh bone marrow specimen (stored at 2-8°C for no more than 24 hours).
2. Sample collection: take 1-3ml of bone marrow cells anticoagulated with heparin sodium
3. Sample preservation: after fixation, the cell suspension shall be stored at -20±5℃ for no more than 12 months; The prepared cell slides can be stored at -20 ±5℃ for no more than 1 month. When the sample storage temperature is too high or too low, or the cell suspension is volatilized excessively or polluted during storage, the sample will not be used for detection.

[Related Reagents]

The following reagents are required for the experiment but not provided in this kit

① 20×SSC, pH 5.3±0.2

Weigh 176g of sodium chloride and 88g of sodium citrate, dissolve in 800mL of deionized water, adjust the pH to 5.3±0.2 at room temperature, and complete to 1 L with deionized water. High-pressure steam sterilization, stored at 2-8°C, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

② 2×SSC, pH 7.0±0.2

Take 100mL of the above 20xSSC, dilute with 800mL deionized water, mix, adjust the pH to 7.0±0.2 at room temperature, complete to 1L with deionized water, stored at 2-8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

③ Ethanol Solution: 70% ethanol, 85% ethanol
Dilute 700ml, 850ml of ethanol with deionized water to 1L. The shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.
④ 0.3% NP-40/0.4xSSC solution, pH 7.0-7.5
Take 0.6mL NP-40 and 4mL 20×SSC, add 150mL deionized water, mix, adjust the pH to 7.0-7.5 at room temperature, with deionized water complete to a volume of 200mL. Stored at 2-8°C, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.
⑤Fixation solution (methanol: glacial acetic acid = 3:1)
Prepare a ready to use fixation solution by mixing thoroughly 30ml of methanol and 10ml of glacial acetic acid.
⑥ 0.075M KCl solution
Weigh 2.8g of potassium chloride, dissolve in 400mL of deionized water and complete to 500mL with deionized water. Stored at room temperature, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.
⑦ Diamidinyl phenylindole (DAPI) counterstain
Use commercially available anti-quenching DAPI counterstain.

[Instructions]
1. Sample collection and slides preparation
① Sample collection: Take 3mL anticoagulated bone marrow cell samples.
② Cell harvest: The bone marrow cells sample is pipetted to the tip of centrifuge tube and centrifuge at 1000rpm for 10 minutes to remove the supernatant.
Place 3mL of anticoagulated peripheral blood in 15 mL centrifuge tube and centrifuge at 300g for 5min. Carefully aspirate the supernatant and resuspend the cells with approximately 500μL of residue.
③ Cell washing: Add 5mL of 1×PBS buffer, mix and resuspend the cell pellet, centrifuge at 300g for 5min, carefully discard the supernatant, and resuspend the cells with about 500μL of the residue; repeat 1 time.
④ Cells hypotonicty: Add 10mL of hypotonic solution pre-warmed to 37°C to each tube and put at 37°C for 15-20min in the hypotonic water bath.
⑤ Cell Pre-fixation: Pre-fixed cells are added to the cell suspension after addition of 1 mL (10% by volume) fixative solution. Gently mix and immediately centrifuge at 300g for 5min, remove the supernatant, and resuspend the cells with 500μL of residual solution.
⑥ Fixation: Slowly add 10 mL of fixative solution to the cell suspension, fix the cells at room temperature for 10 min, centrifuge at 300g for 5 min, and resuspend the cells with about 500 μL of the residue; repeat once (the cells may be fixed several times until the cells are washed and cleaned).
⑦ Cell suspension preparation: Pipet the supernatant and add the appropriate amount of fixative solution to prepare the appropriate cells suspension concentration.
⑧ Slides preparation: Pipet 3-5μl of cell suspension drop onto the slides, put at 56°C for 30min.

2. Slides preparation
① At room temperature with 2×SSC (pH 7.0) solution, rinse the slide 2 times for 5min each time.
② Place the slides in 70% ethanol, 85% ethanol and 100% ethanol for 2min each time, dehydrate and air dry.

3. Denaturation and Hybridization
The following operations should be performed in a darkroom.
① Take the probe at room temperature for 5 minutes. Briefly centrifuge manually (do not use vortex or shaker instrument). Take 10μl droplet in the cell and drop in the hybridization zone, immediately cover 22mmx22mm glass slide area; spread evenly without bubbles the probe under the glass slide covered area and seal edges with rubber (edge sealing must be thorough to prevent dry film from affecting the test results during hybridization).
② Place the glass slide in the hybridization instrument, denature at 88°C for 2 minutes (the hybridizer should be preheated to 88°C) and hybridize at 45°C for 2 to 16 hours.

4. Washing
The following operations should be performed in a darkroom.
① Take out the hybridized glass slides, remove the rubber on the coverslip and immediately place the slides into 2xSSC for 5 seconds, and gently remove the coverslip.
② Place the glass slides in 2xSSC at room temperature.
③ Remove and immerse the slides in a 0.3% NP-40/0.4×SSC solution preheated at 68°C for 2 min.

④ Immerse the glass slides in deionized water at 37°C for 1min, and dry naturally in the dark.

5. Counterstaining

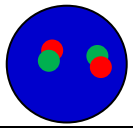
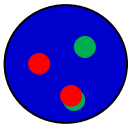
The following operations should be performed in a darkroom

10μl DAPI compound dye is dropped in the hybridization area of the glass slide and immediately covered. The suitable filter is selected for glass slide observation under the fluorescence microscope.

6. FISH results observation

Place the stained slides under a fluorescence microscope and confirm the cells area under a low magnification objective (10×). Under magnification objective (40×) a uniform cells distribution is observed. Then the nuclei FISH results are observed under the high magnification objective (100x).

[Common Signal Type Interpretation]

KMT2A (MLL) gene site 3 signal	KMT2A (MLL) gene site 5 signal
	**Negative**: 2 Fusion
	**Positive**: 1 Orange ; 1 Green 1 Fusion

[Precautions]

① This product is for research use only.
② The results of this kit will be affected by various factors of the sample itself, but also limited by hybridization temperature and time, operating environment and the limitations of current molecular biology technology, which may lead to wrong results.
③ Users must understand the potential errors and accuracy limitations that may exist in the detection process.
④ All chemicals are potentially dangerous. Avoid direct contact and wastes should be properly disposed off.

[Manuscript version and approval date]

Manual version: V1.1 reviewed on 07 December 2021
Approval date: 01 June 2019