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Product Catalogue Number FP-183 **For clinical diagnosis and scientific research.**

E2A Gene Break Apart Probe Detection Kit

[Product Name] E2A Gene Break Apart Probe Detection Kit (Fluorescence In Situ Hybridization Method).

[Product Introduction]

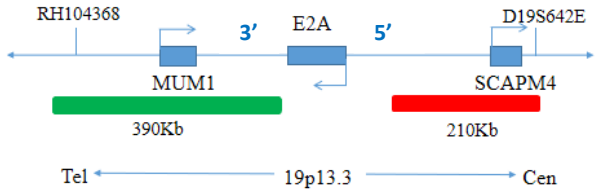
This kit uses Orange fluorescein, Green fluorescein to label E2A probe and bind E2A gene with the target site by in situ hybridization.

[Product Main Components]

The kit consists of E2A dual color probe as shown in Table 1.

Table 1: Kit composition

Package Specifications	Component name	Specifications	Quantity	Main components
10 Tests/box	E2A dual color probe	100μL/Tube	1	(5'Cen) E2A Green probe ; (3'Tel) E2A Orange 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 1-3 mL of heparin sodium anticoagulated bone marrow cell sample.

3. Sample storage: After fixation, the cell suspension can be stored at -20±5°C for no more than 12 months. The prepared cell slides can be stored at -20±5°C for no more than 1 month. When the storage temperature of the specimen is too high or too low, or when the cell suspension is excessively volatile or contaminated during storage, the sample should not be used for testing.

[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.

⑤ **Fixation 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]

Sample collection and slides preparation

① Sample collection: Take 1-3 mL of heparin sodium anticoagulated bone marrow cell sample.
② Cell harvest: Pipet uncultured bone marrow cells or cultured bone marrow cell samples into a 15mL conical centrifuge tube, centrifuge at 500g for 5 minutes, carefully aspirate and discard the supernatant, and leave about 500μL of residual liquid to resuspend the cells.
③ Cell washing: Add 5mL of 1×PBS solution by pipetting to mix and resuspend the cell pellet, centrifuge at 500g for 5min, carefully aspirate and discard the supernatant, keep about 500μL of residual liquid to resuspend the cells; repeat once.
④ Cell permeation: Add 10mL hypotonic solution to each tube (pre-warmed at 37°C bath) and place at 37°C water bath hypotonic for 20min.
⑤ Cell pre-fixation: Add 1mL (10% volume) of fixative to the cell suspension after permeation to pre-fix the cells, gently pipette to mix, and immediately centrifuge at 500g for 5min, and remove the supernatant, keep about 500μL of residual liquid to resuspend the cells.
⑥ Cell fixation: Slowly add 10 mL of fixative to the cell suspension, put at room temperature for 10 min to fix the cells. Centrifuge at 500g for 5 min, and keep about 500μL of residual liquid to resuspend the cells; repeat once (the cells can also be fixed multiple times until the cells precipitate and wash out).
⑦ Preparation of cell suspension: After the last cell fixation and centrifugation, aspirate the supernatant and add an appropriate amount of fixative to prepare the cell suspension with the appropriate concentration.
⑧ Producer: 3-10μL cell suspension was dropped onto the slide and aged at 56 ℃ for 0.5h.
⑨Pretreatment: the slides were rinsed twice in 2×SSC solution at room temperature for 5min each time.
⑩ Dehydration: the cell drops were placed in 70% ethanol, 85% ethanol and 100% ethanol for 2 minutes respectively and then dried naturally.

1. 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.

2. 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 for 1 min.
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.

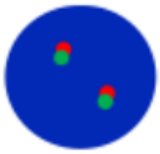
3. Counterstaining

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.

4. FISH results observation

Place the counterstained film under the fluorescence microscope, and first put it under the low-power objective lens (10x) Confirm the cell area under the microscope; Go to 40× Under the objective lens, find a position where the cells are evenly distributed; Then in the high-power objective (100x) The FISH results of nuclei were observed.

[Common Signal Type Interpretation]

E2A signal E2A signal	
	**Negative**: 2 Fusions (2F)
	**Positive**: 1 Orange 1 ; Green 1 Fusion (1R ; 1G ; 1F)

[Troubleshooting]

The common factors influencing test results and the handling methods are shown in Table 2:

Table 2: Frequent problems and handling methods

Problem	Probable cause	Recommended solution
Strong background of slides	Inadequate wash of glass slide before preparation of specimens	Wash the glass slide using the absolute ethyl alcohol.
	Inadequate wash after hybridization	Assure that the wash buffer is prepared in line with Instruction For Use, assure the correct pH value and temperature of wash buffer, remove the coverslip and repeat the washing steps.
	Improper use of filter sets	Replace with suitable filter sets to reduce the background light.
	Improper hybridization condition	Assure the temperature of hybridization instrument is set as 42ºC.
	The temperature is too low when washing	Assure that the wash buffer reaches to the required temperature when washing the slides.
	The washing intensity of wash buffer is too low	Assure the wash buffer is prepared in line with Instruction For Use. (Low SSC concentration or high NP-40 concentration would help improving the washing intensity of wash buffer).
Weak counterstaining	Weak counterstaining	Remove coverslip, at room temperature, immerse the slides in the wash buffer containing 2 × SSC/ 0.1%NP-40 for 5 minutes. And then sequentially immerse the slides in 70%, 85% and 100% ethanol solution for 1 minutes respectively, and then perform the counterstaining.
	The counter stain has been kept under long-term storage or excessive light	Assure the counter stain is stored at -20ºC and protected away from light, assure its effect.
	Inadequate denaturation of specimens	Assure the temperature of hybridization instrument is set as 83ºC, at least 10 minutes in advance is needed to preheat hybridization instrument.

No signal or weak signals	The probe mixture and hybridization buffer were not mixed sufficiently before use	Blow the probe mixture and mix the probe sufficiently, centrifuge for a short time.
	The probe mixture on tissue slides dries too fast	After dropping probe mixture the target area should be covered by coverslip immediately, when washing the slides you can only remove one coverslip at a time, and dip it into wash buffer immediately before removing next coverslip.
	Air bubbles formed under coverslip during hybridization	The coverslip should cover the probe mixture in order to gently squeeze out air bubbles.
	Inappropriate hybridization condition	Ensure to comply with the time and temperature required by hybridization and do not leave gaps when sealing the slides with rubber cement. The hybridization time should be adjusted according to the situation.
	Improper wash buffer or incorrect washing conditions	Be sure to follow the requirements of Instruction for Use to formulate the wash buffer. Ensure that the temperature of wash buffer reaches to the temperature predetermined in washing step. The thermometer and pH meter should be accurately calibrated. Remove coverslip before immersing the slide into wash buffer.
	Inappropriate storage of probe or specimens slides	Make sure that the probe mixture is stored at -20ºC and protected from light. Place the slides without hybridization at -20ºC for long-term storage or at room temperature for short-term storage. Place the hybridized slides at -20ºC, away from light, and store for less than 6 months.
	Incorrect use of DAPI counter stain, excessively high brightness of counter stain	Remove the coverslip, immerse the slides in 2 × SSC/ 0.1%NP-40 for 5 minutes at room temperature. Sequentially immerse the slides in 70%, 85% and 100% ethanol solution for 1 minutes respectively, and then perform the counterstaining after air drying the slides.
	Inappropriate filter sets were selected for observation	Use correct filter sets to observe the probe fluorescence. For the detailed information, please consult the technical service department of Wuhan HealthCare Biotechnology Co., Ltd.

If there are other problems, please contact our technical support at: cs@healthcare-bio.com

[Precautions]

1. Please read this manual carefully before testing. The testing personnel shall receive professional technical training. The signal counting personnel must be able to observe and distinguish orange red and green signals.
2. When testing clinical samples, if it is difficult to count the hybridization signals and the samples are not enough to repeat the retest, the test will not provide any test results. If the amount of cells is insufficient for analysis, again, the test will not provide test results.
3. The formamide and DAPI counterstaining agent used in this experiment have potential toxicity or carcinogenicity, so they need to be operated in the fume hood and wear masks and gloves to avoid direct contact.
4. The results of this kit will be affected by various factors of the sample itself, but also limited by enzyme digestion time, hybridization temperature and time, operating environment and limitations of current molecular biology technology, which may lead to wrong results. The user must understand the potential errors and accuracy limitations that may exist in the detection process.
5. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical wastes and should be properly disposed of.
6. This product is for clinical diagnosis and scientific research.

[Manuscript version and approval date]

Manual version: V1.1 reviewed on 07 December 2021

Approval date: 12 April 2019