

E2A Gene Break Apart Probe Detection Kit (CW-183)

Intended use

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

Product composition

The kit consists of E2A dual color probe (100 µL/Tube).

Storage condition

Keep sealed away from light at $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$. 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 $2-8^{\circ}\text{C}$ in dark. For long-term preservation after opening, keep the lid sealed at $-20^{\circ}\text{C}\pm 5^{\circ}\text{C}$ 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 $2-8^{\circ}\text{C}$ 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 $-20\pm 5^{\circ}\text{C}$ for no more than 12 months. The prepared cell slides can be stored at $-20\pm 5^{\circ}\text{C}$ 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

1. **20×SSC, pH 5.3±0.2**

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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 $2-8^{\circ}\text{C}$, 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 20×SSC, 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 $2-8^{\circ}\text{C}$, 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-40/0.4×SSC 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 $2-8^{\circ}\text{C}$, 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-queenching DAPI counterstain.

Sample collection and slides preparation

1. Sample collection: take 1-3ml of heparin sodium anticoagulant bone marrow cells.
2. Cell harvesting: suck the uncultured bone marrow cells or the cultured bone marrow cell samples into a 15ml tip bottom centrifuge tube, centrifugate 500g for 5min,

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carefully suck and discard the supernatant, and leave about 500µl residual liquid to suspend the cells again.

3. Cell washing: add 5ml of 1×PBS buffer solution, blow and mix up the heavy suspension cell precipitation, centrifugate 500g for 5min, carefully suck and discard the supernatant, and leave about 500µl of residual solution to heavy suspension cell; repeat once.
4. Cell hypotonic: add 10ml of hypotonic solution to each tube (37°C warm bath in advance), and water bath at 37°C hypotonic for 20min.
5. Cell pre fixation: add 1ml (10% volume) of fixed solution to the cell suspension after hypotonic treatment, gently blow and mix, centrifugate 500g immediately for 5min, remove the supernatant, and leave about 500µl of residual solution for cell suspension.
6. Cell fixation: slowly add 10ml of the fixed solution to the cell suspension, leave it at room temperature for 10min to fix the cell, centrifugate 500g for 5min, and leave about 500µl of the residual solution to re suspend the cell; repeat once (or fix the cell several times until the cell is precipitated, washed and cleaned).
7. Preparation of cell suspension: after the last centrifugation of cell fixation, the supernatant is sucked off, and a proper amount of fixed solution is added to make cell suspension with appropriate concentration.
8. Preparation: take 3-10µl cell suspension drop to slide, aging at 56°C for 0.5h.
9. Pretreatment: 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.

Denaturation and hybridization

The following operations need to be carried out in the darkroom.

1. Take out the probe, leave it at room temperature for 5min, turn it upside down with force, mix it well, and then centrifuge it for a short time (no vortex instrument vibration). Take 10µl drop in the cell hybridization area, and immediately cover the cover glass of 22mm×22mm. The probe should be evenly expanded under the cover glass without bubbles, and then seal the edge with rubber glue (the edge must be completely sealed to prevent the dry piece from affecting the test results in the hybridization process).
2. The cell drops were placed on the hybridizer and denatured at 88°C for 2 min (the hybridizer should be preheated to 88°C) and hybridized at 45°C for 2-16h.

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-40/0.4xSSC 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.

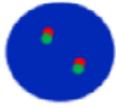
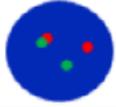
Counterstaining

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.

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 40x 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.

 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 the below table.

Frequent problems and solutions

Problem	Possible 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 42°C
	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 -20°C and protected away from light, assure its effect
No signal or weak signals	Inadequate denaturation of specimens	Assure the temperature of hybridization instrument is set as 83°C, at least 10 minutes in advance is needed to preheat hybridization instrument
	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 -20°C and protected from light. Place the slides without hybridization at -20°C for long-term storage or at room temperature for short-term storage. Place the hybridized slides at -20°C, 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

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]

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