MDS Chromosome and Gene Abnormality Kit
(In Situ Hybridization)
Instruction For Use

[Product Name]

MDS Chromosome and Gene Abnormality Kit (In Situ Hybridization)

[Specification]

5 Tests/box, 10 Tests/box, 20 Tests/box.

[Intended Use]

This reagent is used for In Situ Hybridization staining based on routine staining(Such as: HE staining) to provide auxiliary information for physicians in diagnosis.

Myelodysplastic Syndromes (MDS) is a type of clonal myeloid disease originating from hematopoietic stem cells. It is characterized by ineffective hematopoiesis, refractory cytopenia, and high risk of transforming into acute myeloid leukemia (AML). Typical chromosomal abnormalities involved in the MDS Vienna diagnostic criteria include (-5, -5q, -7, -7q, +8, -20q, -Y). Deletion of the long arm of 5q is the most common rearrangement in acute myeloid leukemia (AML) and MDS. It occurs in more than 20% of MDS, and chromosome 5 abnormalities account for more than 40% of treatment-related MDS. The deletion of the entire chromosome 7 or the deletion of the entire long arm 7q is a recurrent abnormality of MDS, which often indicates that the patient has a poor prognosis and is easy to develop into leukemia. Abnormality of chromosome 8 (single or multi-body) can occur in a variety of diseases, and is closely related to the blast phase of myeloid cells and the increase of basophils. 20q deletion can be seen in about 6% of primary MDS. Tumor suppressor genes located in this region are prone to heterozygous or homozygous deletions. Patients with -20/20q- have a better prognosis. The Y chromosome counting probe can be used for prenatal diagnosis and monitoring of the effect of bone marrow transplantation for sex chromosome mismatches, and its appearance in MDS alone indicates a better prognosis.

"WHO Tumor Classification Series-Pathology and Genetics of Hematopoietic and Lymphoid Tissue Tumors" divides cytogenetic changes as a prognostic factor into 3 groups: Good prognosis

(low-risk group): cytogenetics is normal, del(5q), del(20q) and -Y are individually abnormal; Poor prognosis (high-risk group): complex cytogenetic abnormalities, that is, ≥3 recurring abnormalities, or abnormal chromosome 7; Poor prognosis (high-risk group): complex cytogenetic abnormalities, that is, ≥3 recurring abnormalities, or abnormal chromosome 7; Therefore, the use of interphase cells to perform multi-target detection from the cytogenetic level can assist in the diagnosis of MDS and the judgment of prognosis, thereby assisting in guiding the choice of clinical treatment options.

[Principle of Detection]

Fluorescent In Situ Hybridization (FISH) enables a specific nucleotide fragments in cells clearly displayed by fluorescence. Fluorescently labeled DNA probes can bind to the target sequence to emit fluorescence, and the DNA fragments after hybridization can be directly observed under a fluorescence microscope. The formalin-fixed and paraffin-embedded tissue is placed on the slide, and the DNA is first denatured into single-stranded DNA, and then combined with the probe DNA in an orderly manner. After hybridization, unbound probes are washed away, the excess probes are washed away, and at the same time, the cell nucleus is stained with the counterstain 4',6-diamidino-2-phenylindole (DAPI) to emit blue fluorescence. The hybridized probe can be observed under a fluorescence microscope.

[Main Components]

Contains EGR1/D5S72 probe(Contains Fluorescence-labeled probes, formamide, SSC, dextran sulfate), CEP7/D7S486 probe(Contains Fluorescence-labeled probes, formamide, SSC, dextran sulfate), CEP8/D20S108 probe(Contains Fluorescence-labeled probes, formamide, SSC, dextran sulfate) and DAPI counterstain solution(Contains DAPI, anti-fading liquid, glycerin).

Components not included but necessary for the test:

Blood (bone marrow) cell sample pretreatment kit (fluorescence in situ hybridization method) (Contains hypotonic buffer, pepsin solution, washing buffer I, washing buffer II and pepsin powder)

Other reagents not provided:

Xylene;Ethanol (anhydrous, 85%, 70%); Methanol; glacial acetic acid; Deionized water; Rubber glue.

[Storage and Validity]

stored at -20℃±3℃ away from light, sealed conditions, valid for 12 months.

Transportation conditions: the transportation temperature does not exceed 8°C, No more than a week.

[Applicable Instrument]

Fluorescence in situ hybridization system;

Fluorescence microscope, in which:

Objective lens: It is recommended to use 100 times achromatic objective lens of immersion type, which can obtain satisfactory results.

Immersion Oil: Low level self-fluorescence Immersion Oil exclusive use for fluorescence microscopy.

Optical Filters: It is recommended that customers use the probe to find out the details of the filter set used by the filter set supplier in order to select the filter suitable for labeled fluorescent stains.

Green fluorescence: excitation wavelength is 496nm , and emission wavelength was 520nm.

Red fluorescence: excitation wavelength was 551nm ,and emission wavelength was 572nm.

[Specimen Requirements]

Bone marrow or peripheral blood (2-3ml), heparin anticoagulant, the sample size depends on the specific situation of the patient, if the patient with aplastic anemia has fewer white blood cells, should be increased collection.

Treatment within 2 hours after collection, specimen transport and preservation is recommended at 2-8℃, no more than 72h.

[Test Method]

1. Test equipment

Fluorescence in situ hybridization system, Fluorescence microscope, Centrifuge, water bath, induction cooker, etc.

2. Reagent preparation

2.1 Ethanol solution (70% ethanol, 85% ethanol, 90% ethanol)

Dilute 700mL, 850mL, and 900mL of absolute ethanol with purified water to 1000mL, respectively, and store at 2°C-8°C during use. The reagent should be discarded after 6 months of preparation or after hybridization of 20 slides. If the reagent is turbid or contaminated, it should also be discarded.

2.2 Gastric digestive juice

Add 1 tube of pepsin powder (0.1g) to every 50mL pepsin solution, and put it in a 37°C±1°C constant temperature water bath for use.

2.3 Fixative

Prepare cell fixative at the ratio of methanol (AR): glacial acetic acid (AR)=3:1, and the cell fixative is now ready for use.

3. Test method

3.1. Cell collection and preparation

3.1.1. Take 2～3ml of peripheral blood or bone marrow (sodium heparin anticoagulation) and centrifuge at 2000rpm for 5 minutes, remove the supernatant;

3.1.2. Add 10ml low-osmolar buffer, and treated in the thermostatic water bath for 20min at 37℃±1℃.

3.1.3. Add 1ml fixative,fix for 10min at room temperature, centrifuged at 2000rpm for 10min, and remove the supernatant.

3.1.4. The cells were resuspended with 10ml fixative, centrifuged at 2000rpm/min for 10min, remove supernatant , repeat this step three times.

3.1.5. Single-cell suspension was prepared, then prepare cell slides and aged for 30min.

3.2 Slide processing

3.2.1. Put the slides into the preheated pepsin solution and digest for 2-10min.

3.2.2. Rinse the slides with purified water twice,5 min/ time.

3.2.3. Take out the slide, process the slides by 70%, 90% and 100% ethanol in sequence for 2 min, dry the slides at room temperature.

3.3 Denaturation and hybridization of sample and probe (be careful to avoid light)

3.3.1. Remove the PML/RARA probe from the refrigerator, centrifuge instantly, and add 10μL to the target area with a pipette, Cover the specimen with coverslip to avoid bubbles.

3.3.2. Mount the slide with rubber cement along the edge of the cover glass. Pay attention to the complete mounting to prevent the hybridization solution from evaporating.

3.3.3. The slides were placed on the hybridization instrument and were denatured at 83°C for 6 min according to the set program, and hybridized at 40°C overnight.

3.4 Wash after hybridization1(Keep away from light)

3.4.1. 30min before washing, put the prepared washing buffer I in a water bath and preheat to 72 ±1℃.

3.4.2. Take out the slide glass, remove the rubber glue, and incubate the slide glass in washing buffer I at room temperature for 5 minutes to remove the cover glass.

3.4.3. Put the slides in 72±1℃ Washing Buffer I for 2min;

3.4.4. Remove slide, and put slide into washing buffer I at room temperature for 1min.

3.4.5.Remove slide, and wash the slides in 70% and 85% ethanol solution for 2min in turn and dry them.

3.5 DAPI counterstain solution staining and mounting

Add 10μL DAPI to the target area of the slide and cover with a coverslip, Apply nail polish to the edges of the coverslip, incubate at -20℃ in dack for 10-20 min.

3.6 Microscopic examination:

3.6.1. Place the slides in an environment of -20℃ for 15 minutes before observing.

3.6.2 Take the slide out of the -20℃ environment and observe under the fluorescence microscope

[Reference range]

Collected 20 normal human peripheral blood cells, each counted 200 cells, the abnormal threshold was: D5S721(5) and EGR1(5q) were missing 5%; chromosome 7 and D7S486(7q) were missing 5%; No. 8 multisomy 3%; D20S108 fragment single copy 5%.

Threshold = mean (i) + 3 x standard deviation (SD).

[Explanation of test results]

1. The cells should be randomly counted in the samples.
2. Counting cells must have clear and recognizable signals in each channel.
3. Do not analyze areas with uneven hybridization.
4. Do not analyze if the outline of the nucleus is unclear or overlaps.

5. Do not analyze the area where the background depth affects the signal judgment.

6. Counting results shall be independently completed by two participants, and the results should be consistent before confirmation.

7. If the signal in over 25% of the nucleus is too weak, the region should not be analyzed.

8. If signals are present in more than 10% of the cytoplasm, the region should not be analyzed.

9. First determine the adequacy of the slide hybridization

Nuclear morphology: The boundary of the cell nucleus is usually clear, and the cell nucleus is in a complete form;

Background status: The background should not contain particulate matter that affects the count;

Fluorescence signal intensity: The signal should be bright, obvious and easy to calculate. The signal should exist in bright light, oval shape. Avoid excessively scattered signals.

10. Fluorescence signal observation of target area.

Use a 40× or 100× objective lens and a suitable filter to observe the probe signal. Adjust the focus to observe the size and shape of the target signal and noise (non-target hybridization signal). Ensure that the background does not have a strong fluorescent interference signal.

Scan the entire slices to observe the overall fluorescence signal distribution of the cells, and select a more representative area for counting.

11. Select cells in the target area and count

Select an area with better cell nuclei distribution (such as the area that can distinguish a single cell nucleus), and ensure that the selected area can represent the observed signal distribution characteristics; start to analyze the cells and record the fluorescent signal characteristics of each cell; repeat the above operations until Count up to 500 cells;

12. Signal counting rules

Adjust the focus to find the signal in the nucleus. Analyze the selected cells with clear boundaries.

[Method Limitations]

This kit uses fluorescence in situ hybridization to detect abnormal numbers of myelodysplastic syndromes D5S721, EGR1, D7S486, D20S108, and chromosomes 7 and 8. It cannot be used to detect other gene mutation methods.

[Product Performance]

1. Fluorescence signal intensity

After the probe is hybridized with a pair of Control slice of lymphocytes cultured in peripheral blood and a sample of myelodysplastic syndrome, under a fluorescent microscope, both should emit a fluorescent signal that can be recognized by the naked eye.

2. Probe sensitivity

In the EGR1/D5S721 combination, 100 chromosome 5 in 50 metaphase cells were analyzed, and at least 98 of them showed a green fluorescent signal of EGR1 labeled by 1 gene locus and an orange fluorescent signal of D5S721 labeled by a gene locus.

In the Cep7/D7S486 combination, 100 chromosomes of 50 metaphase cells were analyzed, and at least 98 of them showed a green fluorescent signal marked by a centromere and an orange fluorescent signal marked by a gene locus of D7S486.

In the Cep8/D20s108 combination, 100 chromosomes 8 and 20 of 50 metaphase cells were analyzed, and at least 98 of them showed a centromeric marker of green fluorescence signal of chromosome 8 and a gene locus marker D20s108 Orange fluorescent signal.

3. Probe specificity

A pair of photographs of lymphocytes cultured in peripheral blood on cells in metaphase,

In the EGR1/D5S721 combination, 100 chromosomes of 50 metaphase cells were analyzed. At least 98 chromosomes showed their specific fluorescent signals in their respective gene locus regions.

In the Cep7/D7S486 combination, 100 chromosomes of 50 metaphase cells were analyzed. At least 98 chromosomes showed their specific fluorescence signals in the centromeric region and the gene locus region.

In the Cep8/D20s108 combination, 100 chromosomes 8 and 20 of 50 metaphase cells were analyzed, and at least 98 of them showed their specific fluorescence signals in the centromeric region and the gene locus region.

[Cautions]

1. This kit is an in vitro diagnostic reagent and can be reused.
2. During the experimental operation of this kit, it is necessary to wear latex gloves to avoid contact with the skin. In case of accidental contact, rinse immediately with plenty of water.
3. The discarded samples and experimental wastes during the experiment shall be recycled and treated as medical waste.
4. In order to obtain ideal results, it is necessary to ensure that the reagents are correctly prepared and stored in accordance with the instructions.

[Symbols]

Symbol	Used for	Symbol	Used for

	The date by which the device should be used		Any special operating instructions
LOT	Batch code	IVD	In vitro diagnostic medical device
	Temperature limit		Name and address of manufacturer
CE	CE mark	EC REP	Authorized representative in the European Community
	Keep away from sunlight	REF	Reference number
NON STERILE	Non-sterile		

[Basic Information]

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