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For Professional Use Only

Bladder Cancer Cells chromosome and gene anomaly probe detection kit Instructions Manual

CE IVD

[Product Name] Bladder Cancer Cells chromosome and gene anomaly probe detection kit

[Package Specifications] 10 Tests/box

[Intended Usage]

Bladder cancer is the most common malignant tumor of the urinary system. In the course of bladder cancer development, the abnormal expression of the cancer cells chromosome karyotype is extremely complex. Studies have shown that a considerable number of non-random chromosomes number and structure aberrations occur in the development, staging, grading, and therapeutic response of bladder cancer. A large number of studies have shown that chromosomes 3, 7, 17, and 9p21 are aberrations main diagnostic marker of bladder cancer, and detecting these abnormal chromosomes has important significance in the diagnosis and prognosis of bladder cancer.

The recurrence rate of bladder cancer patients is higher after treatment, therefore it should be monitored. Standard monitoring protocols include cystoscopy and urine exfoliative cells examination. Cystoscopy is invasive and has poor compliance. It is difficult to diagnose early superficial tumors, and 10%-30% is false negative. Although the urine exfoliative cells examination is not invasive, it has high sensitivity to high-grade tumor, but it has low sensitivity to low grade tumor. Therefore, finding an effective early detection method for bladder cancer recurrence is an urgent problem to be solved. A large number of reports have shown that urine exfoliated cells fluorescence in situ hybridization technology has the advantages of being non-invasive, sensitive and specificity, and the sensitivity increases with the increase of tumor staging stage, and is an ideal means for diagnosing and monitoring bladder cancer recurrence.

This product uses urinary sediment cells from patients with suspected bladder cancer as a test object, and uses fluorescence in situ hybridization to detect loss of aneuploidy and p16 (9p21) on chromosomes 3, 7, 17 in exfoliated cells. It can be used as an auxiliary measure for the early diagnosis of bladder cancer and the recurrence of bladder cancer in the patients with hematuria.

[Detection Principle]

The kit is based on fluorescence in situ hybridization and uses nucleic acids probe labeled with fluorescein. The target gene to be detected is homologously complementary to the nucleic acids probe used. Both are denatured, annealed, and renatured. A hybrid of the target gene and the nucleic acids probe is formed, and the qualitative, quantitative or relative positioning analysis of the gene to be measured under the microscope is performed by the fluorescence detection system. This kit utilizes a rhodamine fluorescein (RHO)-labeled orange-colored probe and a green fluorescein isothiocyanate (FITC)-labeled green probe. The two probes can be bound to the target detection site by in situ hybridization.

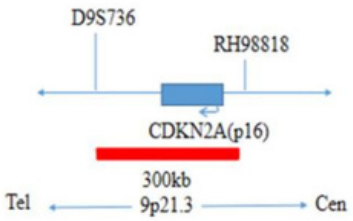
This kit provides two sets of probes (CEP3/CEP7, p16/CEP17). Under normal conditions, each set of probes is displayed as two orange red signals and two green signals under a fluorescence microscope. When there is a missing chromosome or gene in the target sequence, the green or orange signal decreases accordingly. Detection of 3, 7, 17 aneuploidy and deletion of p16 (9p21) by this method can be used as an auxiliary detection method for clinical diagnosis and postoperative recurrence of bladder cancer.

[Product Composition]

The kit consists of one of CEP3/CEP7, P16/CEP17dual color probe, as shown in Table 1.

Table 1 Kit composition

Component name	Specifications	Quantity	Main components
CEP3/CEP7 dual color probe	100μl/Tube	1	CEP3 Orange probe; CEP7 Green probe
P16/CEP17 dual color probe	100μl/Tube	1	P16 Orange probe; CEP17 Green probe



[Storage conditions & Validity]

Keep sealed away from light at -20℃±5℃. The product is valid for 20 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℃ in dark. For long-term preservation after opening, keep the lid sealed at -20℃±5℃ away from light. The kit is transported below 0℃.

[Applicable Instruments]

Fluorescence microscopy imaging systems including fluorescence microscopy and filter sets suitable for DAPI, Green, and Orange.

[Sample Requirements]

Applicable specimen types: Fresh urine deposited cell specimens stored for no more than 2 hours.

[Test Method]

1. Related reagents

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

①20×SSC,pH5.3±0.2

Sodium chloride	176g
Sodium citrate	88g

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 2~8ºC, 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 2~8ºC, 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

NP-40	0.6mL
20xSSC	4mL

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 2-8ºC, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

⑤ Fixative solution (Methanol: Glacial acetic acid = 3:1)

Fill the flask with 30mL of methanol and 10mL of glacial acetic acid and mix thoroughly for immediate use.

⑥PBS buffer, pH 7.4±0.2

Sodiumchloride	8g
Potassiumchloride	0.2g
Monosodiumhydrogenphosphate	3.58g
Potassiumdihydrogenphosphate	0.27g

Dissolve the reagents in 800mL of deionized water, adjust the pH to 7.4±0.2 at room temperature, and complete to 1 L 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 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.

⑧ HCl Solution

Measure 8.2ml of concentrated HCl; mix with deionized water to 100mL to obtain 1M HCl solution and store at room temperature. According to the need, dilute to 0.01M using the 10 times dilution method.

⑨ Di-amiindyl phenyl indole (DAPI) dyeing agent

Please use commercially available DAPI counterstains containing anti-quencher.

2. Sample collection and slides preparation

①Sample collection: Collect morning urine 200mL (within 2h) and place it in a 50ml tip centrifuge tube and centrifuge at room temperature 1800rpm for 10min.

②Cells harvest: Carefully discard the supernatant (often pours precipitate, and if precipitation is small, use a pipette to carefully aspirate), add 2 mL of preheated hypotonic solution (0.075M KCl) along the tube wall, carefully blow heavy sediment, and continue to add hypotonic solution for a final volume of 10 mL, in hypotonic solution for 20 minutes at 37°C in a water bath.

③After hypotonic treatment, 2 mL of the fixative solution is added gently in the preparation by shaking while adding.

④ Immediately centrifuge at room temperature at 1800rpm for 10min and discard the supernatant.

⑤Use fresh fixation solution to re-suspend precipitation and place 10min at room temperature.

⑥ At room temperature centrifuge at 1800rpm for 5min and discard the supernatant.

⑦Re-suspend the pellet with 1 mL of fresh fixation solution and collect the resuspension in a 1.5mL EP tube. Centrifuge on a palm centrifuge for 3 min and discard the supernatant.

⑧ According to the amount of precipitation, add the suitable amount of fresh fixation solution to re-suspend the precipitation (pellet).

⑨Drops are examined microscopically to adjust the cell concentration.

⑩ Droppieces.

3. Slides processing

①Slides are baked at 56°C for 30 minutes.

②. Soak 5 min×2 times in 2×SSC buffer.

③37°C pepsin solution (0.04%) and digest for 10-15 min.

d). Soak 5 min×2 times in 2×SSC buffer.

④Dehydrate in gradient alcohol 70%, 85%, 100%.

4. Denaturation and Hybridization

The following operations should be performed in a darkroom.

① Take out the CEP3/CEP7 and p6/CEP17 probes and put at room temperature for 5min. Mix and centrifuge briefly (do not vortex). 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 slides 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.

5. 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 immerse the slides in a 2xSSC solution for 5 seconds and remove the coverslip.

② Place the slides in a 2×SSC at room temperature for 1 min.

③ Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC solution and wash for 2min. (Preparation of 0.3% NP-40/0.4xSSC: For 1L preparation, take 3mL NP-40 and 20mL 20xSSC, dissolve fully, mix well, and use 1M NaOH to adjust the pH to 7.2).

④ Remove the slides and immerse in a 37°C preheated deionized water, wash for 1min and dry the slides naturally in the dark.

6. Counterstaining

The following operations should be performed in a darkroom.

Dip 10-15μL of DAPI counterstain into the hybridization area of the glass slide, immediately cover, and then use the suitable filter to observe the sections under the fluorescence microscope.

7.FISH results observation

Place the counterstained film under the fluorescence microscope, and first put it under the low-power objective lens (10 ×) 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 (100 ×) The FISH results of nuclei were observed.

[FISH cells signal type determination]

Determine the signal type of each group of probes in interphase cells.

Probe combination 1: p16/CEP17 Marker color: Orange/Green

Common abnormal types: p16 deletion or amplification; chromosome 17 aneuploidy

Normal signal mode: 2 Orange / 2 Green

Abnormal signal mode: 1 Orange red / 2 Green; 0 Orange / 2 Green; 2 Orange / 3 Green; 3 Orange / 3 Green; other

Probe combination 2: CEP3/CEP7 Marker color: Orange/Green

Common Abnormal Types: Aneuploidy on chromosomes 3 and 7

Normal signal mode: 2 Orange / 2 Green

Abnormal signal mode: 3 Orange / 2 Green; 2 Orange / 3 Green; 3 Orange / 3 Green; other

[Positive value determination or Reference interval]

1. A sample of urinary bladder epithelial exfoliated cells was collected from 20 patients with non-bladder cancer or normal controls.

2. Using the above method steps to prepare slides and perform FISH experiments;

3. Threshold determination: 100 cells were observed for each sample combination of each probe, and the mean and standard deviation of the percentage of cells showing abnormal signal types were calculated. The abnormal threshold was defined as the mean + 3 times the standard deviation. Abnormal threshold = mean (M) + 3 x standard deviation (SD).

For example: p16 deletion or amplification determination.

Twenty (20) cases of non-bladder cancer patients or normal human were selected for urine samples to establish a threshold. After cell treatment, p16 FISH was performed. 100 cells were observed in each sample, and the cell types and their corresponding cell percentage were counted.

For example, p16 gene detection abnormal threshold establishment.

Table 2 Abnormal threshold establishment

No	Sample1	Sample2	……	Sample20	AverageValue	SD	Threshold(%)
Cellcounting	100	100	……	100			
Abnormalcells(Zerocopy%)	1	1	……	3	2	1.500	6.5
Abnormalcells(Singlecopy%)	2	1	……	0	1.35	1.492	5.8
Abnormalcells(≥3copies)	1	0	……	0	0.4	0.821	2.9

For each sample, analyze 100 cells per probe and use the threshold to determine the result:

1. The detection index is greater than the threshold, and it is determined as Positive;

2. The detection index is less than the threshold, and it is determined as Negative;

3. The detection index is equal to the threshold, increase the number of cells in the test sample to determine the final result;

4. When there are abnormalities on two or more chromosomes or multiple abnormalities on the same chromosome, it indicates the existence of bladder cancer cells.

[Test method limits]

This kit uses fluorescence in situ hybridization to detect chromosomal or gene abnormalities in CEP7/CEP3 and p16/CEP17 cells, and cannot be used for detection of single base mutation.

[Product performance index]

1. Fluorescence signal strength

After the probe effective hybridization with the karyotype reference material, the probe should emit fluorescence signals that can be identified by the naked eye under the fluorescence microscope.

2. Sensitivity:

2.1 The sensitivity of CEP3 orange probe was analyzed in 100 chromosome of chromosome 3 in metaphase division of 50 cells, and at least 98 of chromosome 3 showed 1 orange red fluorescence signal.

2.2 The sensitivity of CEP7 green probe was analyzed in 100 chromosome of chromosome 7 in metaphase division of 50 cells, and at least 98 of chromosome 7 showed 1 green fluorescence signal.

2.3 The sensitivity of CEP7 green gene probe was analyzed in 100 chromosome of chromosome 17 in metaphase division of 50 cells, and at least 98 of chromosome 17 showed 1 green fluorescence signal.

2.4 The sensitivity of p16 orange probe was analyzed in 100 chromosome of chromosome 9 in metaphase division of 50 cells, and at least 98 of chromosome 9 showed 1 orange-red fluorescence signal.

3. Specificity:

3.1 The specificity of CEP3 orange probe was analyzed in 100 chromosome of chromosome 3 in metaphase division of 50 cells, and at least 98 of chromosome 3 showed 1 specific orange red fluorescence signal in the target area.

3.2 The specificity of CEP7 green probe was analyzed in 100 chromosome of chromosome 7 in metaphase division of 50 cells, and at least 98 of chromosome 7 showed 1 specific green fluorescence signal in the target area.

3.3 The specificity of CEP17 green probe was analyzed in 100 chromosome of chromosome 17 in metaphase division of 50 cells, and at least 98 of chromosome 17 showed 1 specific green fluorescence signal in the target area.

3.4 The specificity of p16 orange probe was analyzed in 100 chromosome of chromosome 9 in metaphase division of 50 cells, and at least 98 of chromosome 9 showed 1 specific orange-red fluorescence signal in the target area.

[Precautions]

1. This product is only used for in vitro diagnosis.

Please read this manual carefully before testing. The testing personnel shall receive professional technical training, and the signal counting personnel must be able to observe and distinguish orange and green signals.

3. When testing clinical samples, when it is difficult to count the hybridization signal and the sample is not enough to repeat the retest, the test will not provide any test results. If the amount of cells is insufficient for analysis, the test will not provide test results.

4. Formamide and DAPI counterstaining agent used in this experiment have potential toxicity or carcinogenicity. It is necessary to operate in the fume hood and wear masks and gloves to avoid direct contact.

5. 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 the limitations of current molecular biology technology, which may lead to wrong chromosome or gene abnormality results. Users must understand the potential errors and accuracy limitations that may exist in the detection process.

6. All chemicals are potentially dangerous. Avoid direct contact. Used kits are clinical waste and should be properly disposed of.

[Reference]

[1]. WilliamsonMPetal.p16(CDKN2)isamajordeletiontargetat9p21inbladdercancer.HumMolGenet.1995Sep.4(9):1569-77.

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[4]. Dun Wenchao, Gu. Shenyang Research advances in the detection of chromosomal aberrations in bladder cancer [J] Modern Medicine 2013, 8, 41(8).

[Basic information]

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