Wuhan HealthCare Biotechnology Co., Ltd.
Building #8, Optics Valley Precision Medicine Industry Park Phase 1, #9 Gaokeyuan 3rd. Road, East Lake High-Tech Zone, 430206 Wuhan City, Hubei Province, PEOPLE'S REPUBLIC OF CHINA.

For Professional Use Only

MDS chromosome and gene anomaly probe detection kit Instructions Manual

[Product Name] MDS chromosome and gene anomaly probe detection kit

[Package Specifications] 10 Tests/box

[Intended Use]

This kit uses fluorescence in situ hybridization to detect the absence/deletion of chromosomes 5, 7, 20 long arm and chromosomes 5, 7, 8, Y chromosomal anomalies in patients with myelodysplastic syndrome. The test sample is bone marrow cells.

Myelodysplastic syndrome (MDS) is a group of heterogeneous diseases that are generally considered to originate from hematopoietic stem cells and belong to malignant clonal diseases. Studies have shown that 40% to 60% of patients with MDS have non-randomized chromosomal anomalies, of which - the most common are 5/5q-, -7/7q-, +8, 20q-, and -Y. Among the common chromosomal anomalies' in patients with MDS, some chromosomal anomalies have specific diagnostic value. Immunosuppressive therapy is effective in some patients with simple +8, 20q-, or Y-; karyotype analysis also has important value in the classification, treatment, and prognosis of MDS, such as single Y-, 5q- or 20q- patients' have a good prognosis, while patients with complex chromosomal anomalies (≥3 anomalies) or chromosome 7 anomalies have a poorer prognosis. Patients with other anomalies have a moderate prognosis. These anomalies are of great significance in the diagnosis, treatment, and prognosis of MDS.

This kit was not clinically combined with gene targeting therapy, and was validated only for the performance of gene detection. This kit is only suitable for the detection of myelodysplastic syndromes and provides physicians with supplementary diagnostic information.

[Detection Principle]

Fluorescence in situ hybridization is a technique that directly observes specific nucleic acids in cells in vitro. According to the principle of base pairing, the specific DNA sequence is complementary to the target sequence in the cell. Since the probe is fluorescent, the hybridization probe and the target DNA can be clearly observed under the fluorescence microscope due to the fluorescence of the probe under the appropriate excitation light.

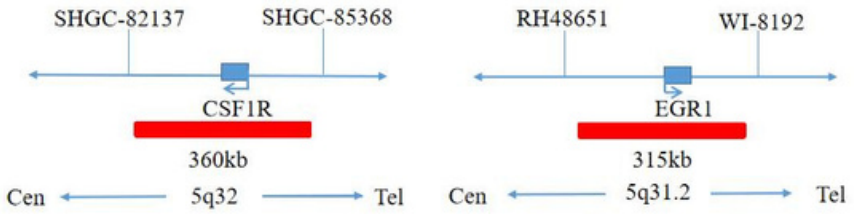
This kit uses orange fluorescent markers CSF1R、EGR1、D7S486、D7S522、D20S108、CEPY、CEPX, and CEPY probes. The D5S630、CEP7、CEP8、CEPX、Yq12 probes are labeled with green fluorescence, and the probes are bound to the target detection site by in situ hybridization. Under normal conditions (no gene deletions and chromosomal anomalies), two orange-red signals and two green signals are shown under a fluorescence microscope. When there is a deletion of a gene, a green or orange signal is lacking, and when there are multiple chromosomes, the probe signal increases. Detection of gene deletions and chromosomal abnormalities by this method is of great importance in the diagnosis, treatment, and prognosis of MDS.

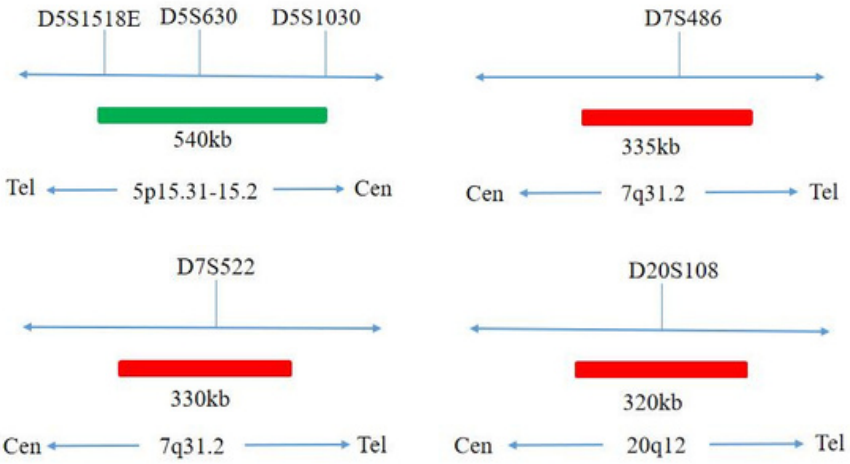
[Product Composition]

This kit consists of one of CSF1R/D5S630、EGR1/D5S630、D7S486/CEP7、D7S522/CEP7、D20S108/CEP8、CEPY/CEPX、Yq12/CEPX probe hybridization solutions as shown in Table 1.

Table 1 Kit composition

Cat#	Component name	Specifications	Quantity	Main components
FP-011-1	D7S486/CEP7 dual color probe	100μl/Tube	1	D7S486 orange probe, CEP7 green probe
FP-011-2	D7S522/CEP7 dual color probe	100μl/Tube	1	D7S522 orange probe, CEP7 green probe
FP-011-3	CSF1R/D5S630 dual color probe	100μl/Tube	1	CSF1R orange probe, D5S630 green probe
FP-011-4	EGR1/D5S630 dual color probe	100μl/Tube	1	EGR1 orange probe, D5S630 green probe
FP-011-5	D20S108/CEP8 dual color probe	100μl/Tube	1	D20S108 orange probe, CEP8 green probe
FP-011-6	CEPY/CEPX dual color probe	100μl/Tube	1	CEPY orange probe, CEPX green probe
FP-011-7	Yq12/CEPX dual color probe	100μl/Tube	1	CEPX orange probe, Yq12 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 under 0℃.

[Applicable Instruments]

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

[Sample Requirements]

1. Applicable specimen types: Fresh bone marrow specimens stored at 4°C for less than 24 hours and used for FISH detection after culture.
2. When specimens are stored at too high or too low a temperature (eg, frozen), the specimen will not be used for testing and should be discarded.
3. Bone marrow cell suspensions for karyotype analysis should be stored at -20°C for FISH detection.
4. If the cell suspension is excessively volatile or contaminated during storage, the sample should be discarded.

[Testing Method]

1. Related reagents

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

①. 20×SSC (sodium citrate buffer), pH 5.3±0.2

Sodiumchloride	176g
Sodiumcitrate	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 2~8℃, 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℃, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

③. 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 2~8℃, the shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

④. 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.

⑤. PBS buffer, pH 7.4±0.2

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

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

water. High-pressure steam sterilization, stored at 2~8℃, the solution shelf life is of 6 months. Discard if the reagent appears cloudy (turbid) or contaminated.

⑥. 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.

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 concentrated HCL, mix with deionized water and complete to 100ml, store at room temperature to obtain 1M HCL. Based on needs dilute to 0.01M by 10-fold 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

①. Sample collection: Heparin anticoagulant bone marrow.

② Cells harvest: The uncultured or cultured bone marrow cells sample is pipetted to the tip of centrifuge tube and centrifuge at 1000rpm for 10 minutes to remove the supernatant.

③ Low permeability:

Wash cells once with PBS, centrifuge at 1000rpm for 10 minutes, and pipet the supernatant. Add 0.075mol/L KCL solution (6~8mL) pre-warmed at 37°C, mix with a pipette, and store in an incubator at 37°C for 20 to 30 minutes.

④Pre-fixation:

Add 2mL of 3:1 methanol, glacial acetic acid fixative solution and mix evenly. Centrifuge at 1000rpm for 10min.

⑤Fixation:

Aspirate the supernatant; add freshly prepared 5mL of 3:1 methanol - glacial acetic acid fixative solution, mix evenly, fix for 10 min, centrifuge at 1000 rpm for 10 min.

⑥Repeat step ⑤ twice.

⑦Cells suspension preparation:

Pipet the supernatant and add the appropriate amount of fixative solution to prepare the appropriate cells suspension concentration.

⑧Slides preparation:

Use a pipette to gently stir the cell suspension, then blot it off and place it onto a clean, fat-free glass slides soaked in ethanol. Drip 1 to 2 drops per slide, put at 56°C for 30min.

The prepared slides can be stored in a refrigerator at 4°C or in a refrigerator at -20°C for about 1 to 4 weeks.

3. Slides processing

①At room temperature, rinse the glass slides twice with SSC (pH 7.0) solution for 5min each time.

②Place the glass slides in 70% ethanol, 85% ethanol and 100% ethanol and dry for 2 minutes.

③ Perform the hybridization experiment according to the hybridization procedure.

4. Denaturation and Hybridization

The following operations should be performed in a darkroom.

① Take out the pre-pared 6 slides and mark them. Take out the CSF1R/D5S630, EGR1/D5S630, D7S486/CEP7, D7S522/CEP7, D20S108/CEP8, CEPY/CEPX, Yq12/CEPX seven-tube dual-color probes, briefly centrifuge after mixing. The probes sunk to the bottom of the tube, and take 10μL of each and drop on the seven slides hybridization zone. Immediately cover the 22mmx22mm glass slide area; spread evenly without bubbles the probe under the glass slides covered area and seal edges with rubber (edge sealing must be thorough to prevent dry film from affecting the test results during hybridization).

②Place the glass slides in the hybridizer, denature at 88°C for 2 minutes (the hybridizer should be preheated to 88℃) and hybridize at 45°C for 2 to 16 hours.

5. Washing

The following operations should be performed in a darkroom.

①Use tweezers to carefully tear off the cover glue around the slides. Avoid sticking off or moving the cover glass. Immerse the slides in 2×SSC for about 5 seconds. Take out and gently push the cover glass to the edge of the slides with tweezers. Use tweezers to gently remove the cover

slide.

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

③Take out the slides and immerse in a preheated at 68°C 0.3% NP-40/0.4xSSC solution, 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 deionized water preheated at 37°C for 1 min. 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 glass slide under the fluorescence microscope and under the natural light, first under the low power objective lens (10×) to confirm the cell area under the microscope; Go to 40× Under the objective lens, find a position where the cells are evenly distributed; At high power objective (60×、 100×) select cells with complete nuclear boundary, uniform DAPI staining, no overlapping nuclei and clear signals. At least 200 cells should be randomly selected to count the orange, green and yellow signals in the nucleus.

[Positive Value Determination or Reference Interval]

1. Signal classification and counting

.①Normal cells signal: There are 2 orange-red signals and 2 green signals in single interphase nuclei (one orange-red signal and one green signal in single interphase nuclei after CEPY/CEPX hybridization).

②Anomaly signals:

CSF1R/D5S630: 1 orange-red signal, 2 green signals, 5q deletion in a single interphase nucleus, 1 orange-red signal, 1 green signal, chromosome 5 deletion;

EGR1/D5S630: There are 1 orange-red signal, 2 green signals, 5q missing in single interphase nucleus, 1 orange-red signal, 1 green signal, chromosome 5 is missing;

D7S486/CEP7: There is 1 orange-red signal, 2 green signals, and 7q deletion in single interphase nucleus; there is 1 orange-red signal and 1 green signal in single interphase nucleus, and chromosome 7 is missing;

D7S522/CEP7: There is 1 orange-red signal, 2 green signals, and 7q deletion in single interphase nucleus. There is 1 orange-red signal and 1 green signal in single interphase nucleus, chromosome 7 is missing;

D20S108/CEP8: There are 1 orange-red signal, 2 green signals, and 20q deletion in single interphase nucleus; there are 2 orange-red signals, 3 green signals, and chromosome 8 trisomy in single interphase nucleus;

CEPY/CEPX: There is only one green signal in a single interphase nucleus, and the Y chromosome is missing.

Yq12/CEPX：There is only one green signal in a single interphase nucleus, Yq12 is missing.

Randomly count 200 cells, and the number of normal signal cells and two different abnormal signal cells are counted. Each cell is counted once and only cells with a hybridization signal (both color signals) are counted. No signal or only a single color signal is not counted (except for CEPY/CEPX, Yq12/CEPX), and weak or over dispersed cells are not counted.

2. FISH results determination

Abnormal results detection requires anomaly threshold establishment.

①Anomaly threshold

- It is recommended to select 20 patients with non-leukemia or normal human bone marrow samples as negative controls.
- Use the above method to prepare the slides for FISH experiments.
- Anomaly threshold setup: Each sample is analyzed for 200 cells. The percentage of abnormal signals in each probe group is counted. The mean and standard deviation of the percentage of cells showing abnormal signal patterns is calculated. The abnormal threshold is defined as mean +3 x standard deviation. **Abnormal Threshold = Mean (M) + 3 × Standard Deviation (SD)**

Example: Table 2: 20 non-leukemia patients or normal subjects were selected as negative controls for FISH detection.

Table 2: Abnormal threshold setup

No.	Abnormal cells (%)

Sample1	5
Sample2	3
……	…..
Sample20	4
Averagevalue	3
SD	0.3
ThresholdValue	(Anomalythreshold=Averagevalue+3xSD)3.9

②Results determination

If the detection value of cells number showing abnormal signal is greater than the anomaly threshold, it is determined as a Positive result. If the detection value of cells number showing the abnormal signal is less than the anomaly threshold, it is determined as a Negative result. If the detection value of cells number showing abnormal signal is equal to the anomaly threshold, increase the counting to 500 cells the number of observations of the sample cells, to determine the final result.

Taking Table 2 as an example, if the percentage of the abnormal signal cells in the CSF1R gene FISH test of the sample was more than 3.9% the anomaly threshold (for example 8%), the patient had 5p deletion. If the percentage of the abnormal signal cells in the CSF1R gene FISH test showed that the percentage of the abnormal signal cells was less than 3.9% the anomaly threshold (for example: 2% In this case, there was no 5q deletion in the patient. The results of other gene loci are determined the same as above.

[Test Method Limits]

This kit is for fresh bone marrow cells and is not recommended for use in other cells. Any change may alter the results of the test; this kit is only for the deletion detection of chromosomes 5, 7, and 20 long arm. The detection of chromosome 5, 7, 8 and Y cannot be used for the detection of a single base mutation, and cannot be used as the sole basis for the treatment, prognosis, or other clinical management of patients with myelodysplastic syndromes. A comprehensive assessment based on the patient's medical history and other diagnostic results is required.

[Product Performance Index]

1. Fluorescence signal strength : After the probe effective hybridization with the karyotype reference material, the probe should emit fluorescence signals which can be identified by the naked eye under the fluorescence microscope.

2. Sensitivity:

2.1 The sensitivity of CSF1R gene probe was analyzed in 100 chromosome of chromosome 5 in metaphase division of 50 cells, and at least 98 of chromosome 5 showed 1 orange red fluorescence signal.

2.2 The sensitivity of D5S630 locus probe was analyzed in 100 chromosome of chromosome 5 in metaphase division of 50 cells, and at least 98 of chromosome 5 showed 1 green fluorescence signal.

2.3 The sensitivity of EGR1 gene probe was analyzed in 100 chromosome of chromosome 5 in metaphase division of 50 cells, and at least 98 of chromosome 5 showed 1 orange-red fluorescence signal.

2.4 The sensitivity of D7S486 locus probe was analyzed in 100 chromosome of chromosome 7 in metaphase division of 50 cells, and at least 98 of chromosome 7 showed 1 orange-red fluorescence signal.

2.5 The sensitivity of CEP7 locus 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.

2.6 The sensitivity of D7S522 locus probe was analyzed in 100 chromosome of chromosome 7 in metaphase division of 50 cells, and at least 98 of chromosome 7 showed 1 orange-red fluorescence signal.

2.7 The sensitivity of D20S108 locus probe was analyzed in 100 chromosome of chromosome 20 in metaphase division of 50 cells, and at least 98 of chromosome 20 showed 1 orange-red fluorescence signal.

2.8 The sensitivity of CEP8 probe was analyzed in 100 chromosome of chromosome 8 in metaphase division of 50 cells, and at least 98 of chromosome 8 showed 1 green fluorescence signal.

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

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

2.11 The sensitivity of Yq12 probe was analyzed in 100 chromosome of chromosome Y in metaphase division of 50 cells, and at least 98 of chromosome Y showed 1 green fluorescence signal.

2.12 The sensitivity of CEPX probe was analyzed in 100 chromosome of chromosome Y in metaphase division of 50 cells, and at least 98 of chromosome X showed 1 orange-red fluorescence signal.

3. Specificity:

3.1 The specificity of CSF1R gene probe was analyzed in 100 chromosome of chromosome 5 in metaphase division of 50 cells, and at least 98 of chromosome 5 showed 1 specific orange red fluorescence signal in the target area.

3.2 The specificity of D5S630 locus probe was analyzed in 100 chromosome of chromosome 5 in metaphase division of 50 cells, and at least 98 of chromosome 5 showed 1 specific green fluorescence signal in the target area.

3.3 The specificity of EGR1 gene probe was analyzed in 100 chromosome of chromosome 5 in metaphase division of 50 cells, and at least 98 of chromosome 5 showed 1 specific orange-red fluorescence signal in the target area.

3.4 The specificity of D7S486 locus 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.

3.5 The specificity of CEP7 locus 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.

3.6 The specificity of D7S522 locus 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.

3.7 The specificity of D20S108 locus probe was analyzed in 100 chromosome of chromosome 20 in metaphase division of 50 cells, and at least 98 of chromosome 20 showed 1 specific orange-red fluorescence signal in the target area.

3.8 The specificity of CEP8 centromeric probe was analyzed in 100 chromosome of chromosome 8 in metaphase division of 50 cells, and at least 98 of chromosome 8 showed 1 specific green fluorescence signal in the target area.

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

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

3.11 The specificity of Yq12 centromeric probe was analyzed in 100 chromosome of chromosome Y in metaphase division of 50 cells, and at least 98 of chromosome Y showed 1 specific green fluorescence signal in the target area.

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

[Precautions]

1. This product is only used for in vitro diagnosis.
2. 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 number 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, so they need to be operated 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]

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[Basic information]

Name of registrant / manufacturer: Wuhan HealthCare Biotechnology Co., Ltd.

Address:Building #8, Optics Valley Precision Medicine Industry Park Phase 1, #9 Gaokeyuan 3rd. Road, East Lake High-Tech Zone, 430206 Wuhan City, Hubei Province, PEOPLE'S REPUBLIC OF CHINA

Contact: 027-87570662

Name of after-sales service unit: Wuhan HealthCare Biotechnology Co., Ltd.

Production address: Building #8, Optics Valley Precision Medicine Industry Park Phase 1, 9 Gaokeyuan 3rd. Road, East Lake High-Tech Zone, 430206 Wuhan City, Hubei Province, PEOPLE'S REPUBLIC OF CHINA

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Manufacturer

Wuhan HealthCare Biotechnology Co., Ltd.

Building #8, Optics Valley Precision Medicine Industry Park Phase 1, #9 Gaokeyuan 3rd. Road, East Lake High-Tech Zone, 430206 Wuhan City, Hubei Province, PEOPLE'S REPUBLIC OF CHINA

E-mail: cs@healthcare-bio.com; Website: www.healthcare-biotech.com

European Representative

Kingsmead Service B.V.

Zonnehof 36, 2632 BE, Nootdorp, Netherland

Resnova S.r.l
Via dei Colli S.Paolo 3/D – 00072 ARICCIA (RM)- Italy
Tel.: **(+39)0693955058** – Fax.: **(+39)0693955059**
E-mail: **servizioclienti@resnovaweb.it** – PEC mail: **resnova@pec.it**
Per invio ordini: **ordini@resnovaweb.it** – Web site: **www.resnovaweb.com**