Instruction for Use

【Product Name】

Cytokeratin Fragment 19 (eCLIA)

【Order Information】

REF NO.	Package Size
0320501501	50T
0320501502	2×50T
0320501503	100T
0320501504	2×100T

【Intended Use】

Immunoassay for in vitro quantitative determination of Cytokeratin Fragment 19 （CYFRA21-1）in human serum and plasma.

Cytokeratin (CK) is a subunit of intermediate filament, one of the structural proteins that form epithelial cells. With a relative molecular weight of about 40KD, it is the smallest member of the keratin family. It is widely distributed on the surface of normal tissues, such as alveoli, esophagus, bladder and other epithelial cells. So far, 20 different cytokeratin polypeptide chains (CK1-CK20) have been identified. The complete cytokeratin polypeptide chain is poorly soluble, but soluble protein fragments in serum can be detected. [1, 2, 3, 4]

Cytokeratin fragment 19 is a soluble fragment of cytokeratin reference CK19. It can be specifically bound to two monoclonal antibodies KS19.1 and BM19.21, hence called CYFRA21-1.[5] Under normal condition, CYFRA21-1 exists in the form of oligomer with very low content. When epithelial cells become cancerous, activated proteases accelerate cell degradation, releasing large amounts of cytokeratin fragments into the blood and increasing CYFRA21-1 level.

CYFRA21-1 is one of the preferred markers for non-small cell lung cancer, especially for squamous cell carcinoma, and it has auxiliary diagnostic value. Blood CYFRA21-1 also shows positive rates of different degrees in other malignant tumors, such as bladder cancer, esophageal cancer, nasopharyngeal cancer, ovarian cancer and cervical cancer. It shows certain increase in some benign diseases, such as hepatitis, cirrhosis, pancreatitis, pneumonia and tuberculosis, but the positive rate is relatively low. Renal failure can also lead to an increase in CYFRA21-1.

Blood CYFRA21-1 is an important prognostic indicator for non-small cell lung cancer. Continuous increase of the serum CYFRA21-1 indicates poor prognosis.

Serum CYFRA21-1 can be used to monitor the curative effect of non-small cell lung cancer. The continuous increase of CYFRA21-1 concentration indicates the disease progression. Serum CYFRA21-1 can be used for follow-up and recurrence monitoring of non-small cell lung cancer. Generally, the detection should be conducted once every 3 months within 2 years after treatment, and once every 6 months within 3 to 5 years. .

【Principles of the testing method】

Sandwich principle. Total duration of assay: 9 minutes.

The Cytokeratin Fragment 19 (CYFRA21-1) Assay Kit adopts the double-site sandwich electrochemiluminescence method. CYFRA21-1 in the sample, biotinylated CYFRA21-1 monoclonal antibody and the ruthenium (Ru) complex-labeled CYFRA21-1 monoclonal antibody form an antigen-antibody sandwich complex, which is bound to streptavidin coated magnetic particles, and then transferred to the measuring cell where the magnetic particles are captured onto the surface of the electrode, while the unbound substances are washed away. Chemiluminescence is generated after the electrode is electrified, and the generated optical signal is measured by a photomultiplier, processed by an instrument, and the concentration of CYFRA21-1 in the sample is calculated based on the calibration curve.

【Main Components】

The reagent pack consists of MB, RA, RB, calibrators and control materials (optional), and different components and reagents of lot numbers should ne be used interchangeably..

Components	Ingredients	Volume (50T)	Volume (2×50T)	Volume (100T)	Volume (2×100T)
(MB)	Streptavidin coated magnetic particles, 0.45 mg/mL; 0.1M phosphate-buffered saline (PBS); ProClin300	1×1.8mL	2×1.8mL	1×3.5mL	2×3.5mL
(RB)	Biotinylated CYFRA21-1 antibody, 0.8 mg/L; 0.1M phosphate-buffered saline (PBS); ProClin300	1×3.5mL	2×3.5mL	1×7.0mL	2×7.0mL
(RA)	Ru complex-labeled CYFRA21-1 antibody, 1.0 mg/L; 0.1M phosphate-buffered saline (PBS); ProClin300	1×3.5mL	2×3.5mL	1×7.0mL	2×7.0mL
Calibrator (High)	CYFRA21-1 antigen, 0.1M phosphate-buffered saline (PBS); ProClin300	1×1.0mL	2×1.0mL	1×1.0mL	1×1.0mL
Calibrator (Low)	CYFRA21-1 antigen, 0.1M phosphate-buffered saline (PBS); ProClin300	1×1.0mL	2×1.0mL	1×1.0mL	1×1.0mL
Control Material (High) (Optional)	CYFRA21-1 antigen, 0.1M phosphate-buffered saline (PBS); ProClin300	1×1.0mL	2×1.0mL	1×1.0mL	1×1.0mL
Control Material (Low) (Optional)	CYFRA21-1 antigen, 0.1M phosphate-buffered saline (PBS); ProClin300	1×1.0mL	2×1.0mL	1×1.0mL	1×1.0mL

The assignment process of supporting calibrators in this pack is strictly implemented with reference to GB/T21415, which can be traced to Roche Elecsys CYFRA21-1.

Refer to the quality control card for the target value and range of the quality controls.

Supporting instruments and materials required but not provided in this pack (provided by Lifotronic)

- Auffer
- Buffer
- Concentrated Washing Buffer
- Lifotronics eCL series of eCLIA Systems
- Assay cup
- Sample diluent

【Storage and Shelf Life】

Unopened reagent kit, calibrators and control materials should be placed at 2~8ºC and will be valid for 18 months.

Once opened, they can be stored at 2~8ºC for 56 days, and reagent kit can also be stored in machine (4~15ºC).

The manufacturing date is labeled on the box, kit and bottles, and the expiration time is 18 months after production.

Damaged, expired or contaminated reagents should be discarded.

【Applicable Instrument】

eCL8000 eCLIA System, eCL8000i eCLIA System, eCL8000p eCLIA System, eCL8000x eCLIA System, eCL9000 eCLIA System, eCL9000i eCLIA System, eCL9600 eCLIA System, eCL9900 eCLIA System and eCL9900i eCLIA System.

【Specimen collection, preparation and storage】

It is recommended to use human serum samples or plasma samples collected with EDTA-K2, EDTA-K3, heparin lithium blood collection tubes.

Blood samples should be collected in accordance with the standard operation of venipuncture. After the sample is completely coagulated, centrifugation should be performed to remove residual cellular substances. The sample should be free of air bubbles during testing. Lipid layer covering the sample after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored for 48 hours at room temperature (18~28℃), 4 weeks at 2~8℃, and 6 months at -15℃ or below. Samples should avoid repeated freeze-thaw cycles.

【Testing Procedure】

Testing procedure and precautions

Before testing, the system operation manual of the measuring instrument should be carefully read, so as to obtain relevant information such as system operating procedures, sample management, safety precautions and maintenance, and materials required for testing should be prepared. CYFRA21-1 testing procedures should be called and set up in accordance with the system operating procedures.

Before using the reagent, put it into the analyzer 30 minutes in advance to automatically stir the magnetic bead particles and keep them in suspension.

Recommended environment temperature for testing: 10~30℃; relative humidity: ≤80%.

The CYFRA21-1 test adopts the double-site sandwich electrochemiluminescence method, with the total test time of 9 minutes, and the test method is as follows:

Step 1: After the user applies for testing, the system automatically aspirates 70 μL of biotinylated CYFRA21-1 monoclonal antibody, 70 μL of Ru complex-labeled CYFRA21-1 monoclonal antibody, 35 μL of streptavidin coated magnetic particles and 10 μL of sample into the reaction cup. They are automatically incubated at 37℃ for 9 minutes to form antigen-antibody sandwich complexes, and then the whole complexes are bound to the magnetic particles under the interaction of biotin and streptavidin.

Step 2: After incubation, the system automatically aspirates the reaction mixture into the measuring cell, the magnetic particles are captured onto the surface of electrode, while the unbound substances are washed away by buffer, and the electrodes is applied with voltage to generate chemiluminescence. The generated optical signals are measured by the photomultiplier, and the measured results are automatically determined via the calibration curve specifically generated by the instrument (this curve is obtained by performing two-point calibration for the master calibration curve obtained by reading the reagent RFID).

Calibration

Calibration should be performed using the lot-matching reagent and calibrators.

The target value of the calibrator has been written into the Radio-Frequency Identification card (RFID card) of the kit.

1. Before calibration, the target value, reagent information and master calibration curve information of the calibrator in the RFID card (Radio Frequency Identification, inductive electronic chip or proximity card) of the kit should be imported into the test system by swiping the card.

2. The supporting calibrator is tested, and the analyzer adjusts the master calibration curve according to the test results of the supporting calibrator to obtain the calibration curve tested by the current system (the analyzer can

automatically interpret the validity of the calibration curve according to the adjustment results).

The test system should re-perform the calibration operation under the following conditions:

(1) When different lots of reagents are used;
(2) When the same lot of reagents has been used on the analyzer for more than 28 days;
(3) According to requirements: e.g. if the quality control result exceeds the defined limit.
(4) When changing the buffers of different lot numbers.

Quality Control procedure

In order to ensure the reliability of test results, it is recommended to test the control materials every 24 hours. It is recommended to test the control materials after each calibration, reagent lot replacement, maintenance or troubleshooting. The quality control results should all fall within the defined range. If they exceed the defined range, the reasons such as instrument status, reagents and calibrators should be investigated and the corrective measures should be taken.

Calculation

The system software can automatically calculate the analyte concentration, with the result in ng/mL.

Specimen dilution

CYFRA21-1 concentration in the sample higher than the upper limit of detection can be diluted manually with sample diluent.

The recommended dilution ratio is 1:2. The results are reported by multiplying the measured values by the dilution ratio.

【Biological Reference Interval】

By analyzing the serum samples of 240 healthy population from hospitals, it was calculated that the upper limit of the reference range of 95% is 3.3ng/mL.

Due to the differences in geography, race, gender and age, it is recommended that each laboratory determine the applicability of reference range through tests, and establish the reference range of this laboratory if necessary.

【Interpretation of results】

When interpreting the test results, it is necessary to refer to the patient's overall clinical situation, including: symptoms, medical history as well as other corresponding data and information.

【Limitations】

The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.

The detection range of the kit is 0.1 ng/mL~500 ng/mL. If the CYFRA21-1 concentration in the sample is lower than the lower limit of detection, the result is reported as less than this value (for example, <0.1 ng/mL); if the CYFRA21-1 concentration in the sample is higher than the upper limit of detection, the result is reported as greater than this value (for example, >500 ng/mL), or the sample is manually diluted with sample diluent at the dilution ratio of 1:2, and the result is reported with the measured value multiplied by the dilution ratio.

When jaundice (bilirubin) is ≤65 mg/dL, hemolysis (hemoglobin) is ≤0.5 g/dL, lipemia (triglyceride) is ≤1500 mg/dL, biotin is ≤50 ng/mL, total protein is ≤10 g/dL, and HAMA is ≤100 ng/mL in the sample, the interference deviation of the measured results is within ±10%. When the concentration of rheumatoid factor in the sample is less than or equal to 1000 IU/mL, the relative recovery of the test results is between 90% and 110%.

Eleven kinds of drugs are added to the serum samples containing CYFRA21-1 to make interference samples, and the concentrations of drugs added are as follows: carboplatin (500 μg/ mL), cisplatin (165 μg/mL), docetaxel (1 μg/mL), adriamycin (40 μg/mL), erlotinib (17 μg/mL), etoposide (12 μg/mL), gemcitabine (380 μg/mL), ifosfamide (800 μg/mL), paclitaxel (67 μg/mL) and vinorelbine tartrate (1.23 μg/mL), The CYFRA21-1 contents in the interference sample and the control sample (without drug addition) are measured respectively, and the relative deviation of the measured results is within ±10%.

When the CYFRA21-1 concentration reaches 2000 ng/mL, there is no high-dose hook effect.

【Analytical Performance】

Lower limit of measurement

Limit of Detection (LoD)≤0.1 ng/mL.

Accuracy

The recovery sample added with an appropriate concentration of CYFRA21-1 is tested, and the recovery is between 90% and 110%.

The accuracy control subject to standardized traceability is tested, and the relative deviation of its test results is within ±10%.

Linearity

Within the range of 0.1 ng/mL~500 ng/mL, the correlation coefficient (r) of the kit should not be less than 0.9900.

Within-run imprecision (repeatability)

The coefficient of variation (CV) is less than 6%.

Between-lot imprecision

The coefficient of variation (CV) is less than 10%.

Accuracy of calibrators

The supporting calibrators of the kit are tested, the relative deviation of their test results is within ±10%.

Measured values of quality control

The measured results of supporting assigned quality control of the kit should be within the quality control range.

Homogeneity of calibrator and quality control

Intra-vial homogeneity: Coefficient of variation (CV) ≤ 6%;

Inter-vial homogeneity: Coefficient of variation (CV) ≤ 10%.

【Precaution and Warning】

This kit is only used for in vitro diagnosis;

When using this kit, the relevant operation precautions of the laboratory must be observed;

The test results of this kit can only be used as clinical reference, the patient's clinical evaluation should be based on the patient's clinical symptoms/signs, medical history, other laboratory test results and treatment response, etc.;

Due to methodological reasons or antibody specificity, the results of the same sample tested with reagents of different manufacturers may be different. Therefore, results obtained with different kits should not be directly compared, so as to prevent wrong medical interpretation; it is recommended that the Laboratory Department indicate the characteristics of the reagents in the test report issued to the clinician. If the reagent type is changed during series monitoring, continuous testing should be conducted, and the results should be compared with the original reagent results in parallel to re-determine the baseline value;

This product contains animal-derived substances, and may have potential biological risks. All samples and reaction wastes should be treated as the source of infection, and all wastes must be disposed of in accordance with local regulations.

【Symbol】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Authorized representative in the European Community		In vitro diagnostic medical device
	Use-by date		Indicates this device is in compliance with Europe Directive.
	Batch code		Contains sufficient for<*n*>tests
	Serial number		Biological risks
	Temperature limitation		This way up
	Catalogue number		

【References】

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2. Bodenmueller H. The biochemistry of CYFRA 21-1 and other cytokeratin-tests. Scond J Clin Lab lnvest 1995:;55,Suppl 221:60-66.
3. Stieber P,Dienemann H,Hasholzner U,et al.Comparison of Cytokeratin Fragment 19(CYFRA 21-1) Tissue Polypeptide Atigen(TPA) and Tissue Polypeptide Specific Antigen (TPS) as Tumor Markers in Lung Cancer.Eur J Clin Chem Clin Biochem 1993;31:689-694.
4. Bodenmueller H, Donle F, Kaufmann M, et al. The tumor markers TPA, TPS TPACYK and CYFRA 21-1 react differently with the keratins 8, 18 and 19. Int J Biol Markers1994;9:7-74.
5. Stieber P, Hasholzner U, Bodenmueller H, et al. CYFRA21-1: A new marker in lung cancer. Cancer 1993; 72: 707-713.

【Manufacturer】

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【Version and Issue Date】

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