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 Purpose】

Cytokeratin Fragment 19 (eCLIA) is an immunoassay reagent for in vitro quantitative determination of Cytokeratin Fragment 19 (CYFRA21-1) in human serum and plasma by automated immunassay analyzer to aid diagnosis of non-small cell lung cancer.

【Summary】

Cytokeratin (CK) is a subunit of intermediate filament, one of the structural proteins that form epithelial cells. With a relative molecular weight 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. Nowadays, 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, so CK19 is called CYFRA21-1 too.[5] In a normal condition, CYFRA21-1's existence is in the form of oligomers with extremely low content. When epithelial cells become cancerous, activated proteases accelerate cell degradation and a large number of cytokeratin fragments are released into the blood, leading to CYFRA21-1 increased.

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.

【Principle】

Sandwich principle. Total duration of assay: 9 minutes.

10 μL of sample, biotin labeled 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 Component】

The reagent pack consists of reagent components and CYFRA21-1 Control Material RFID.

Reagent components include MB, RB, RA, CYFRA21-1 Calibrators and CYFRA21-1 Control Materials (Optional). Different reagent components of lot numbers should not be used interchangeably.

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

Traceability: This method has been traced to Roche Elecsys CYFRA21-1.

See the control material value sheet for the target values and ranges of control materials.

【Instruments and Materials Required but Not Provided】

Additional materials for Automated ECL Immunoassay Analyzers eCL8000, eCL8000i, eCL8000p, eCL8000x:

- REF 679005, Auffer, 480 mL, or REF 679004, Auffer, 6×480 mL
- REF 679007, Buffer, 480 mL, or REF 679006, Buffer, 6×480 mL
- [illegible] Concentrated Washing Buffer, 6×1 L
- REF 679011, Assay Cup, 300 T, or REF 679012, Assay Cup, 3000 T

Additional materials for Automated ECL Immunoassay Analyzer eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p:

- REF 0320501804, Auffer, 2×1 L, or REF 0320501805, Auffer, 4×1 L
- REF 0320501904, Buffer, 2×1 L, or REF 0320501905, Buffer, 4×1 L
- REF 0320507502, PreClean, 2×800 mL, or REF 0320507503, PreClean, 4×800 mL
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 032120111, Assay Cup/Assay Tip, 12×2×105

Additional materials for Automated ECL Immunoassay Analyzer eCL9000, eCL9600, eCL9900, eCL9900i:

- REF 0320501801, Auffer, 2×2 L, or REF 0320501806, Auffer, 4×2 L
- REF 0320501901, Buffer, 2×2 L, or REF 0320501906, Buffer, 4×2 L
- REF 0320507501, PreClean, 2×2 L, or REF 0320507504, PreClean, 4×2 L
- REF 0320510001, Assay Cup/Assay Tip, 6×6×105, or REF 0320510002, Assay Cup/Assay Tip, 1×6×105

【Applicable Instrument】

Automated ECL Immunoassay Analyzers: eCL8000, eCL8000i, eCL8000p, eCL8000x, eCL8600, eCL8600i, eCL8600p, eCL8800, eCL8800i, eCL8800p, eCL9000, eCL9600, eCL9900, eCL9900i.

【Storage and Shelf life】

Store at 2ºC~8ºC.

Do not freeze.

Store the reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagents	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks
on the analyzers at 2ºC~15ºC	8 weeks

The Calibrators and Control Materials are stable up to the stated expiration date.

Stability of the Calibrators and Control Materials	
Unopened store at 2ºC~8ºC	18 months
Store at 2ºC~8ºC after opening	8 weeks

【Specimen collection, handling 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. Sample covered by lipid layer after centrifugation should be removed. It is not recommended to use hemolyzed samples.

Samples can be stored 48 hours at 18~28°C, 4 weeks at 2~8°C, and 6 months at -15°C or below. Samples can be repeated freeze-thaw once.

It is recommended to softly shake (Maximum minutes) the reagent bottle to mix the samples. When mixing the sample with the electric oscillator, the time shall not more than 5 seconds. If the mixing time is too long, the obtained signal would be reduced.

【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 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: 10~30ºC relative humidity: ≤80.0%.

Calibration

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

Before calibration, the reagent information should be imported into the analyzer via radio frequency identification reagent card (refer to instrument user manual).

Recalibration is recommended as follows :

(1) When using a new lot of reagent;
(2) When the same lot of reagents has been used on the analyzer for more than 28 days;
(3) If the quality control result exceeds the defined limit;
(4) When changing the buffers of different lot numbers.

Quality Control procedure

For quality control, use CYFRA21-1 control materials.

In addition, other suitable control material can be used.

Control materials for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each

laboratory should establish corrective measures to be taken if values fall outside the defined limits.
If necessary, repeat the measurement of the samples concerned.
Follow the applicable government regulations and local guidelines for quality control.
Please make sure that the correct values are used.

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 negative serum.
The recommended dilution ratio is 1:2 (automatic dilution by instrument or manual dilution).
If manual dilution, the result should be multiplied by the dilution ratio.
If dilution by instrument, the instrument will automatically multiply the result by the dilution ratio.

【Reference intervials】

By analyzing the serum samples of 247 healthy test subjects (male 121, female 126), the limit of 95% reference interval was 3.3 ng/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.

【Limitations】

1. The test results are only for clinical reference and cannot be used alone as the basis for diagnosis or exclusion of cases.
2. The measuring range of the kit is 0.05 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<0.05 ng/mL; if the CYFRA21-1 concentration in the sample is higher than the upper limit of detection, the result is reported as >500 ng/mL (2 × diluted sample is reported as > 1000 ng/mL).
3. When jaundice (bilirubin) is ≤65 mg/dL, hemolysis (hemoglobin) is ≤0.5 g/dL, lipid (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%.
4. When 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) in the sample, the measured results are not affected .
5. When the CYFRA21-1 concentration under 2000 ng/mL, the test results are not be affected by the high-dose hook effect.

【Analytical Performance characteristics】

Detection limit

Limit of Blank = 0.05 ng/mL.
Limit of Detection = 0.1 ng/mL.

Accuracy

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 is not less than 0.9900.

Within-run precision (repeatability)

The coefficient of variation (CV) ≤ 6.0%.

Between-lot precision

The coefficient of variation (CV) ≤ 10.0%.

Measured values of quality control

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

Accuracy of Calibrator

The relative deviation of its test results is within ±10%.

Homogeneity of calibrator and control materials

Within-bottle homogeneity: Coefficient of variation (CV) ≤ 6%.
Between-bottle homogeneity: Coefficient of variation (CV) ≤ 10%.

Analytical specificity

Analog	Concentration (ng/mL)	Result (ng/mL)
CK8	1000	≤0.1
CK18	1000	≤0.1

Method comparison

A comparison of the Lifotronic CYFRA21-1 assay (y) with the Roche Elecsys CYFRA21-1 Method (x) using clinical samples gave the following correlations:
Number of samples measured: 129
Linear regression:
y = 1.0065x+0.5249
r = 0.9946
The sample concentrations were between approx 0.9214 ng/mL to 460.2 ng/mL.

【Precautions and Warnings】

The kit is only used for in vitro diagnostics.
Exercise the normal precautions required for handling all laboratory reagents.
All reagents and samples including specimen, reagents, calibrators and control materials should avoid foaming before and during test.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.
This product contains animal-sourced materials and may have potential biological risk. All samples and test wastes should be treated as the source of infection, and all wastes must be disposed according to local regulations.
Any serious incident that would be occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
The preservative ProClin™300 which contain Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1): May cause an allergic skin reaction. thus, proper personal protection should be adopt while handling the reagent: avoid breathing dust/fume/gas/mist/vapours/ spray, wear protective gloves and contaminated work clothing should not be allowed out of the workplace. If skin irritation or rash occurs: get medical advice/attention, take off contaminated clothing and wash it before reuse, and dispose of contents/container to an approved waste disposal plant

【Bibliography】

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3. Stieber P, Dienemann H, Hasholzner U, et al. Comparison of Cytokeratin Fragment 19 (CYFRA21-1) 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 Markers, 1994, 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.

【Symbols Explanation】

Symbol	Title of Symbol	Symbol	Title of Symbol
	Manufacturer		Consult instructions for use
	Use-by date	IVD	In vitro diagnostic medical device
LOT	Batch code	Σ	Sufficient for <n> tests
SN	Serial number		Biological risks
	Temperature limit		This way up
EC REP	Authorized representative in the European Community	CE	Indicates this device is in compliance with Europe Directive.
REF	Catalogue number	P/N	Material code
	Warning		Date of manufacture
UDI	Unique device identifier		

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