CYFRA 21-1
ENZYME IMMUNOASSAY TEST KIT

Intended use
The Cyfra 21-1 EIA kit is intended for the quantitative determination of soluble cytokeratin 19 fragments in human serum. The assay is to be used as an aid in monitoring disease progression during the course of disease and treatment in lung cancer patients. Serial testing for patient Cyfra 21-1 assay values should be used in conjunction with other clinical methods used for monitoring lung cancer.

Introduction
Cytokeratins are group of intermediate filament proteins and based on the tissue expression, cytokeratins are classified into simple epithelial cytokeratin and stratified epithelial cytokeratins. Cytokeratins expression pattern in the malignant cells are usually retained from the cell of origin, and therefore cytokeratins are being used in tumor typing. Cytokeratin filaments are poorly soluble but following proteolytic degradation, soluble cytokeratin fragments are formed and released into body fluids. Cyfra 21-1 is a soluble fragment of cytokeratin 19 which is an acid type cytokeratin, with a molecular weight of 40kDa. The assumption is that Cyfra 21-1 is released into the bloodstream during cell death, and therefore its level correlates very well with the tumor mass, or more specifically with the necrosis in the tumor, which is a function of the tumor mass.
Elevated levels of cytokeratin 19 fragments are seen in serum from patients with lung cancer and also in other cancers e. g. bladder cancer. In patients with lung cancer, Cyfra 21-1 has been reported to be useful for monitoring the course of disease during treatment and for detection of recurrence.

Test principle
The Cyfra 21-1 Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one monoclonal anti-cytokeratin19 fragments antibody for solid phase (microtiter wells) immobilization and another monoclonal anti-cytokeratin19 fragments antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution.
The standards and test specimen (serum) are added to the anti Cyfra 21-1 monoclonal antibody coated microtiter wells. Then another anti-Cyfra 21-1 antibody labeled with horseradish peroxidase (conjugate) is added. If human Cyfra 21-1 is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the Cyfra 21-1 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 1 hour incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution. The color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of Cyfra 21-1 is directly proportional to the color intensity of the test sample.

Materials and components

Materials provided with the test kits:

- Monoclonal anti- Cyfra 21-1 antibody coated microtiter plate with 96 wells.
- Enzyme conjugate reagent, 12 ml.
- Cyfra 21-1 reference standards containing; 0, 3, 6, 12, 25, and 50 ng/ml of Cyfra 21-1 antigens. 1 set, lyophilized status.
- 50 X Wash Buffer Concentrate, 15 ml.
- TMB Substrate, 12 ml.
- Stop Solution 12ml.
- Control Set. 1 set, lyophilized.

Materials required but not provided:
- Precision pipettes and tips, 0.025ml, 0.05 ml, 0.10 ml, and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Glass tubes or flasks to prepare wash buffer.
- Vortex mixer.
- Absorbent paper or paper towel.
- Microtiter plate reader.
- Graph paper.

Specimen collection and preparation
1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Do not use hyperlipemic, hemolyzed.
4. Avoid turbid and contaminated samples.

Storage of test kits and instrumentation
1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent preparation
1. All reagents should be brought to room temperature (1825°C) and mixed by gently inverting or swirling prior to use. Do NOT induce foaming.
2. Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.
3. If reference standards are lyophilized, reconstitute each standard with 0.5ml distilled water. Allow the reconstituted material to stand at room temperature for at least 20 minutes. Mix them well before assays. Reconstituted standards are stable for up to 20 days when stored sealed at 2-8 °C. For longer period of storage time, aliquot the reconstituted standards in polypropylene tubes and store in -20°C. Do not freeze and thaw more than once.

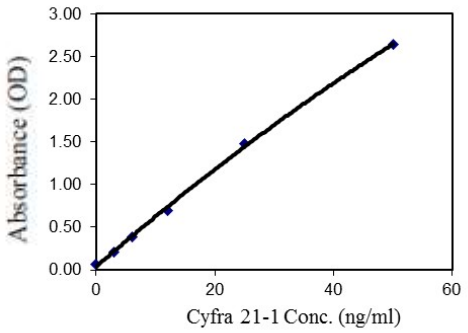
Assay procedure
1. Secure the desired number of coated wells in the holder. Dispense 25µl of Cyfra 21-1 standards, specimens, and controls into the appropriate wells. Gently but thoroughly
mix for 10 seconds.
2. Dispense 100 µl of enzyme conjugate reagent into each well. Mix gently for 30 seconds. It is very important to have a complete mixing in this step. Incubate at room temperature for 1 hour.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
4. Dispense 100µl of TMB substrate into each well. Gently mix for 10 seconds. Incubate at room temperature, in the dark, for 20 minutes.
5. Stop the reaction by adding 100µl of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
6. Read the optical density at 450nm with a microtiter plate reader within 15 minutes.

Important Note:
1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended
4. If a serum specimen contains greater than 50 ng/ml of Cyfra 21-1 the sample must be diluted with "0 ng/ml" standard and re-assayed as described in the assay procedure. Additional "0 ng/ml" standard is available from the manufacturer upon request.

Calculation of results
Calculate the mean absorbance value for each set of Cyfra 21-1 reference standards, specimens and controls. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng per ml on linear graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of Cyfra 21-1 in ng per ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of standard curve
Results of a typical standard run with optical density reading at 450nm shown in the Y axis against Cyfra 21-1 concentrations shown in the X axis.

Cyfra 21-1 Values (ng/ml)	Absorbance (450nm)
0	0.071
3	0.210
6	0.385
12	0.695
25	1.482
50	2.638



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This standard curve is for the purpose of illustration only, and should not be used tocalculate unknowns. Each user should obtain his or her own standard curve and data.

Quality Control

1. It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.
2. The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
3. The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications.

Expected values:

1. It is recommended that each laboratory should determine its own normal and abnormal ranges as to account for its environmental factors such as diet, climate etc.
2. A clinical study of the Cyfra 21-1 Quantitative kit was conducted and results are summarized as follows: Nearly all normal individuals have Cyfra 21-1 values below 3 ng/ml (95 th percentile).
3. The expect ranges are representative only, and do not necessarily reflect the ranges that will be observed in a particular clinical laboratory.

Performance Characteristics:

Precision:

Three concentration levels of Cyfra21-1 samples were used to determine the precision of Cyfra21-1 assay kit. The results were shown in the table below.

Concentrations	NO.	Mean (ng/ml)	S.D.	CV
Level 1	24	3.62	0.11	3%
Level 2	24	15.64	0.79	5%
Level 3	24	32.45	0.64	2%

Limit of Detection:

The sensitivity is defined as the concentration of Cyfra 21-1 antigen that corresponds to the absorbance that is two standard deviations greater than the mean absorbance of 20 replicates of the zero calibrator. The sensitivity is 0.90 ng/ml. The minimum detectable concentration of this assay is estimated to be 1.0 ng/mL.

Detection Range:

The Detection Range is from 1.0 to 50ng/ml without need of sample dilution.

Recovery Test Results:

Various patient serum samples with known Cyfra21-1 were mixed at 1:1 ratio and tested separately. The average recovery was 100.7%.

Cross Reactivity:

The following antigens at high concentrations, as spiked in human serum samples, were assayed to determine the possible reactivity.

Antigens	Concentration	% Cross-react.
PSA	100 ng/ml	< 0.1
AFP	1000 ng/ml	< 0.1
CEA	120 ng/ml	< 0.1
CA125	400 U/ml	< 0.1
CA 15-3	240 U/ml	< 0.1
CA 19-9	240 U/ml	< 0.1
Ferritin	800 ng/ml	< 0.1
Prolactin	240 ng/ml	< 0.1
HCG	1000 mlU/ml	< 0.1

Hook Effect:

No hook-effect has been noticed with samples up to 1000 ng/mL.

Linearity:

Three patient serums were serially diluted with 0ng/ml standard in a linearity study. The average recovery rate (The measured concentration / measured concentration) was 98.3%.

Limitations and applications

1. For diagnostic purposes, the Cyfra 21-1 test results must be used in conjunction with other data available to the physician.
2. The Cyfra 21-1 test should not be used in cancer screening and should not replace any established clinical examination.
3. Samples with Cyfra 21-1 level above 50 ng/ml should be diluted to obtain accurate value.
4. Patients with confirmed lung cancer may have Cyfra 21-1 values in the same range as healthy subjects. Elevated levels of Cyfra 21-1 may also be found in subjects with non-malignant disease e.g. renal failure and certain benign respiratory diseases. Therefore, the level of Cyfra 21-1 cannot be used as absolute evidence for the presence or absence of malignant disease and the Cyfra 21-1 EIA should not be used in cancer screening.
5. The performance of the assay has not been adequately validated in small cell lung cancer (SCLC), large cell carcinoma and Stage I and II lung cancers.
6. Anti-reagent antibodies (human anti-mouse antibody (HAMA) or heterophilic antibodies) in the patient sample may occasionally interfere with the assay, even though specific blocking agents are included in the buffer.

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PRESENTACIÓN:

CONT. CODIGO: RSET060-1