



User's Manual

CYFRA 21-1 ELISA Kit



DEIA6310



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

 Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

 Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)  Fax: 1-631-938-8221

 Email: info@creative-diagnostics.com  Web: www.creative-diagnostics.com

PRODUCT INFORMATION

Intended Use

The CYFRA 21-1 ELISA is an enzyme immunoassay for the quantitative measurement of CYFRA 21-1 in serum or plasma (heparin- or citrate plasma).

General Description

Cytokeratins are epithelial markers whose expression is not lost during malignant transformation. CYFRA 21-1 is a cytokeratin-19 fragment that is soluble in serum and can be used as circulating tumor marker. Although expressed in all body tissues, its major occurrence is in the lung, particularly in lung cancer tissues. CYFRA 21-1 is a sensitive and specific tumor marker of non-small-cell lung cancer (NSCLC), especially of squamous cell subtype (1,2,3). It also reflects the extent of the disease and has an independent prognostic role along with performance status and disease stage in NSCLC (4,5,6). In addition, detection of serum CYFRA 21-1 allows for identification of high risk patients that may benefit from adjuvant chemotherapy (7), and enables the early detection of progressive disease in recurrent NSCLC (8). Additionally, CYFRA 21-1 has been described as a useful marker for esophageal squamous cell carcinoma (9) and for therapy monitoring of bladder cancer (10). The CYFRA 21-1 ELISA uses the two mouse monoclonal antibodies KS19.1 and BM19.21 to determine cytokeratin-19 fragments.

Principles of Testing

The CYFRA 21-1 ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site of the CYFRA 21-1 molecule.

An aliquot of patient sample containing endogenous CYFRA 21-1 is incubated in the coated well with enzyme conjugate, which is an anti-CYFRA 21-1 antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase conjugate is proportional to the concentration of CYFRA 21-1 in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of CYFRA 21-1 in the patient sample.

Reagents And Materials Provided

1. **MTP Microtiter Plate** ,
12 x 8 (break apart) strips, 96 wells; Wells coated with anti-CYFRA 21-1 antibody (monoclonal).
2. **CAL 0-4 LYO Standard (Standard 0 - 4)**,
5 vials, 1 mL each, lyophilized;
Concentrations: 0 - 3 - 10 - 25 - 50 ng/mL
See "Reagent Preparation". Contain non-mercury preservative.

3. **CONTROL LOW LYO & CONTROL HIGH LYO Control Low & High,**
2 vials, 1 mL each, lyophilized;
For control values and ranges please refer to vial label or QC-Datasheet.
See "Reagent Preparation". Contain non-mercury preservative.
4. **SAMPLEDIL Sample Diluent,**
1 vial, 3 mL, ready to use; Contains non-mercury preservative.
5. **ASSAYBUF Assay Buffer,**
1 vial, 7 mL, ready to use; Contains non-mercury preservative.
6. **ENZCONJ Enzyme Conjugate,**
1 vial, 1.2 mL, ready to use, Anti-CYFRA 21-1 antibody conjugated with horseradish peroxidase;
Contains non-mercury preservative.
7. **TMB SUBS TMB Substrate Solution,**
1 vial, 14 mL, ready to use; Tetramethylbenzidine (TMB).
8. **STOP Stop Solution,**
1 vial, 14 mL, ready to use; Contains 0.5 M H₂SO₄,
Avoid contact with the stop solution. It may cause skin irritations and burns.
9. **WASH CONC Wash Solution,**
1 vial, 30 mL (40X concentrated); See "Reagent Preparation".

Note: Additional Sample Diluent for sample dilution is available upon request.

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450 nm \pm 10 nm)
2. Calibrated variable precision micropipettes
3. Absorbent paper
4. Distilled or deionized water
5. Timer
6. Graph paper or software for data reduction

Storage

When stored at 2°C to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2°C to 8°C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for 8 weeks if stored as described above.

Specimen Collection And Preparation

Serum or plasma (heparin- or citrate plasma) can be used in this assay.

The use of EDTA plasma results in increased values.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

1. Specimen Collection

Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma: Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

2. Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2°C to 8°C prior to assaying. Specimens held for a longer time (up to 18 months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

3. Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Sample Diluent and re-assayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µL sample + 90 µL Sample Diluent (mix thoroughly)

b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Sample Diluent (mix thoroughly).

Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

Standards

Reconstitute the lyophilized contents of each vial with 1 mL deionized water and let stand for at least 10 minutes at room temperature. Mix several times before use.

Note: The reconstituted standards are stable for 8 weeks at 2°C to 8°C. For longer storage freeze at -20°C.

Controls

Reconstitute the lyophilized content each vial with 1 mL deionized water and let stand for at least 10 minutes at room temperature. Mix the control several times before use.

Note: The reconstituted controls are stable for 8 weeks at 2°C to 8°C. For longer storage freeze at -20°C.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution. Dilute 30 mL of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 mL. The diluted Wash Solution is stable for 2 weeks at room temperature.

Assay Procedure

General Remarks

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
4. Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.

Test Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 50 µL Assay Buffer into each well.
3. Dispense 10 µL Enzyme Conjugate into each well.
4. Dispense 50 µL of each Standard, Control and samples with new disposable tips into appropriate wells. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for 60 minutes at room temperature.
6. Briskly shake out the contents of the wells. Rinse the wells 3 times with 350 µL diluted Wash Solution per well. Strike the wells sharply on absorbent paper to remove residual droplets.

Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

7. Add 100 µL of Substrate Solution to each well.
8. Incubate for 15 minutes at room temperature.
9. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well.
10. Determine the absorbance (OD) of each well at 450 nm \pm 10 nm with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

Quality Control

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

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. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for

direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

Calculation

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 50 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/mL)	0.05
Standard 1 (3 ng/mL)	0.23
Standard 2 (10 ng/mL)	0.63
Standard 3 (25 ng/mL)	1.37
Standard 4 (50 ng/mL)	2.35

Reference Values

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently healthy adults, using the CYFRA 21-1 ELISA the following data were observed:

Population	n	Mean (ng/mL)	Median (ng/mL)	5 th - 95 th Percentile (ng/mL)	Range (min. - max.) (ng/mL)
Males	121	0.61	0.60	0.10 - 1.33	0.00 - 2.53
Females	119	0.58	0.46	0.00 - 1.38	0.00 - 1.98

Several studies recommended a cut-off concentration of 3.3 ng/mL for CYFRA 21-1, since all patients without disease and 95% of patients with benign lung diseases are found below this value (1,2,3).

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

Detection Range

The range of the assay is between 0.079 ng/mL - 50 ng/mL.

Sensitivity

The Limit of Blank (LoB) is 0.079 ng/mL.

The Limit of Detection (LoD) is 0.185 ng/mL.

The Limit of Quantification (LoQ) is 0.343 ng/mL.

Specificity

The antibodies used for the CYFRA 21-1 ELISA are specific for Keratin 19.

Linearity

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Concentration (ng/mL)	11.60	26.95	45.05	15.55	22.52	37.62
Average Recovery (%)	99.8	103.8	101.8	103.8	104.3	100.1
Range of Recovery (%)	from	95.5	100.8	95.9	101.8	99.8
	to	104.0	108.3	109.1	108.7	107.2

Recovery

Samples have been spiked by adding CYFRA 21-1 solutions with known concentrations in a 1:1 ratio. The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value = (endogenous CYFRA 21-1 + added CYFRA 21-1) / 2; because of a 1:2 dilution of serum with spike material).

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Concentration (ng/mL)	2.24	5.90	10.06	15.98	23.14	38.67
Average Recovery (%)	91.7	94.5	95.4	94.1	94.9	94.8
Range of Recovery (%)	from	87.7	89.1	89.3	91.4	91.3
	to	101.8	105.5	104.1	98.6	100.1

Reproducibility

Intra Assay

The within assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	80	1.90	6.4
2	80	4.31	3.5
3	80	12.68	2.7
4	80	33.89	3.4

Inter Assay

The between assay variability is shown below:

Sample	n	Mean (ng/mL)	CV (%)
1	80	1.90	11.7
2	80	4.31	7.1
3	80	12.68	5.5
4	80	33.89	5.7

Inter-Lot

The inter-assay (between-lots) variation was determined by repeated measurements of samples with 3 different kit lots.

Sample	n	Mean (ng/mL)	CV (%)
1	18	2.62	2.5
2	18	8.68	5.1
3	18	11.07	2.5
4	18	32.75	5.2

Interferences

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 7.5 mg/mL) have no

influence on the assay results.

The assay contains reagents to minimize interference of HAMA and heterophilic antibodies.

Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of CYFRA 21-1 in a sample.

High-Dose-Hook Effect

Hook effect was not observed in this test up to a concentration of 1000 ng/mL of CYFRA 21-1.

Precautions

1. This kit is for in vitro research use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21°C to 26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with Stop Solution containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin,

flush immediately with water.

19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request.

Limitations

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

References

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