Human CYFRA 21-1 ELISA Kit

Enzyme Immunoassay for the quantitative determination of human CYFRA 21-1 in serum and plasma

Catalog number: ARG80897

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

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, particulary 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. It also reflects the extent of the disease and has an independent prognostic role along with performance status and disease stage in NSCLC. In addition, detection of serum CYFRA 21-1 allows for identification of high risk patients that may benefit from adjuvant chemotherapy, and enables the early detection of progressive disease in recurrent NSCLC. Additionally, CYFRA 21-1 has been described as a useful marker for esophageal squamous cell carcinoma and for therapy monitoring of bladder cancer.

The CYFRA 21-1 ELISA uses the two mouse monoclonal antibodies KS19.1 and BM19.21 to determine cytokeratin-19 fragments.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CYFRA 21-1 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any CYFRA 21-1 present is bound by the immobilized antibody. After washing away any unbound substances, HRP-CYFRA 21-1 antibody conjugate is added and

incubate. Then washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of CYFRA 21-1 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of CYFRA 21-1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-antibody Conjugate	1.2 ml	4°C
Standards 0-4 (0, 3, 10, 25, 50 ng/ml)	5 vials	4°C, lyophilized
Control Low	1 vial	4°C, lyophilized
Control High	1 vial	4°C, lyophilized
Sample Diluent	3 ml	4°C
Assay Buffer	7 ml	4°C
40X Wash buffer	30 ml	4°C
Substrate (TMB)	14 ml	4°C
Stop solution	14 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the 40X Wash buffer, Sample Diluent or Assay Buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Avoid using haemolytic, icteric or lipaemic samples and samples contain azide

cannot be assayed.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: Citrate plasma results in decreased data, and EDTA plasma in strongly increased values.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X wash buffer with distilled water to yield 1X wash buffer.
- Standards: Reconstitute the lyophilized contents of the standard vial with 1.0 ml distilled water.
- Controls: Reconstitute the lyophilized content with 1.0 ml distilled water and let stand for 10 minutes in minimum. Mix the controls several times before use.
- Sample: If the initial assay found samples contain CYFRA 21-1 higher than the highest standard, the samples can be diluted with Sample Diluent and then re-assay the samples.
  For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) Dilution 1:10: 10 µL Serum + 90 µL Sample Diluent (mix thoroughly).

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

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

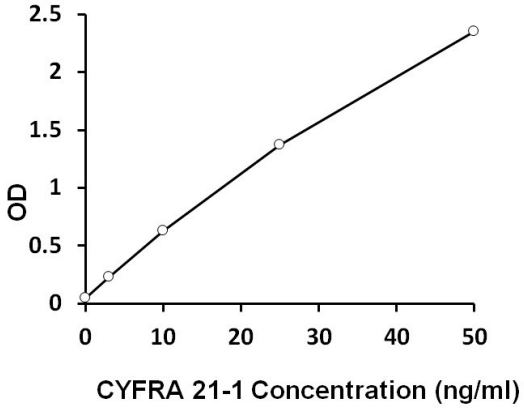
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 µl Assay buffer into each well.
3. Add 10 µl HRP-antibody conjugate into each well.
4. Add 50 µl standards, controls and samples into each well.
5. Cover wells and incubate for 60 minutes at RT.
6. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with wash buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.
7. Add 100 µl of TMB Substrate to each well. Incubate for 15 minutes at room temperature in dark.
8. Add 100 µl Stop solution into each well.
9. Read the OD with a microplate reader at 450 nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, control and samples.
2. Using log-log, semi-log or 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 IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

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



QUALITY ASSURANCE

Sensitivity

The minimal detectable concentration is approximately 0.15 ng/ml.

Specificity

Cross-reactivity with tumor markers CA 15-3, CA 19-9, CA 125, CA 72-4, was not observed.

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 5.5% and the CV value of inter-assay precision was 8%.

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

The assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results.

Recovery

89.6-102.7%