CYFRA 21-1 ELISA Kit

Catalog Number KA4024
96 assays
Version: 03

Intended for research use only

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Introduction

Intended Use

A solid-phase enzyme immunoassay for the quantitative determination of antigen CYFRA 21-1 in blood serum or plasma.
This kit is designed for measurement of antigen CYFRA 21-1 in blood serum or plasma. The kit contains reagents sufficient for 96 determinations and allows to analyze 42 unknown samples in duplicates.

Background

Antigen CYFRA 21-1 is an established name for the epitopes expressed on soluble fragments of cytokeratin 19. Cytokeratin 19 and related cytokeratin 8 molecules are the members of cytokeratin family proteins with molecular weight range ca 25 to 45 kDa which are ubiquitously expressed in all connective tissue cells. Some tumors show elevated production of cytokeratin 19 which in turn may result in increased serum CYFRA 21-1 levels. Most important examples are squamous cell carcinomas (SCC) of the lung and bladder carcinomas. Therefore, the determination of CYFRA 21-1 antigen in patients’ serum or plasma may help in monitoring of tumor growth and efficiency of anti-cancer therapy.

Principle of the Assay

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal antibody to soluble cytokeratin 8/19 (antigen CYFRA 21-1)-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Second antibodies – murine monoclonal antibody to soluble cytokeratin 8/19 (antigen CYFRA 21-1), labelled with peroxidase enzyme, are then added into the microwells. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYFRA 21-1 EIA strips: polystyrene microwells coated with murine monoclonal antibody to soluble cytokeratin 8/19 (antigen CYFRA 21-1).</td>
<td>96 (8x12) wells</td>
</tr>
<tr>
<td>Calibrator set (1-5): Calibrator 1 (6 mL, colourless). The set contains 5 calibrators: 0; 3; 10; 25; 50 ng/mL. Human antigen CYFRA 21-1 diluted in phosphate buffered horse serum, casein solution, preservative – 0.1% phenol; also contains red dye.</td>
<td>6 mL x 1 0.8 mL x 4</td>
</tr>
<tr>
<td>Control serum: Dilution of preselected human serum, with high content of antigen CYFRA 21-1 with casein solution; preservative – 0.1% phenol, colourless.</td>
<td>0.8 mL</td>
</tr>
<tr>
<td>Conjugate: Aqueous solution of murine monoclonal antibody to soluble cytokeratin 8/19 (antigen CYFRA 21-1) coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative and bright red dye.</td>
<td>6 mL</td>
</tr>
<tr>
<td>Substrate solution: Ready-to-use single-component tetramethylbenzidine (TMB) solution.</td>
<td>14 mL</td>
</tr>
<tr>
<td>Washing solution concentrate 26x: Aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative.</td>
<td>22 mL</td>
</tr>
<tr>
<td>Stop solution: 5.0% vol/vol solution of sulphuric acid.</td>
<td>14 mL</td>
</tr>
<tr>
<td>Plate sealing tape</td>
<td>2 slides</td>
</tr>
</tbody>
</table>

Storage Instruction

Store the whole kit at +2…+8 °C upon receipt until the expiration date. After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

Materials Required but Not Supplied

- Distilled or deionized water;
- Automatic or semiautomatic multichannel micropipettes, 100–250 µL, is useful but not essential;
- Calibrated micropipettes with variable volume, range volume 25–250 µL;
- Dry thermostat for 37 °C ±0.1 °C;
- Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0
**Precautions for Use**

- For professional use only.
- This kit is intended for research use only.
- INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including human samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.
- Avoid contact with stop solution containing 5.0% H₂SO₄. It may cause skin irritation and burns.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.
- Do not use the kit beyond the expiration date.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microplate readers.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.
- Do not mix reagents from different lots.
- Replace caps on reagents immediately. Do not swap caps.
- Do not pipette reagents by mouth.
- Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.
Assay Protocol

Reagent Preparation

✓ All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25 °C) before use.
✓ All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
✓ It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.
✓ Prepare washing solution from the Washing solution concentrate 26x by 26 dilutions in distilled water.

Sample Preparation

✓ Specimen Collection and Storage
This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.
Specimens may be stored for up to 48 hours at +2…+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/thawing should be avoided.

✓ Quality Control
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.
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.
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.
**Assay Procedure**

✓ **Procedure Note**

*It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.*

1. Put the desired number of microstrips into the frame; allocate 12 wells for the calibrators (1–5) and control serum and two wells for each unknown sample. **DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.**
2. If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using (zero calibrator). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.
3. Pipet 50 µL of calibrators (1-5) and unknown samples into the wells.
4. Dispense 50 µL of Conjugate into the wells. Cover the wells by plate adhesive tape.
5. Incubate 60 minutes at 37 °C.
6. Prepare washing solution by 26x dilution of washing solution concentrate 26x by distilled water. Wash the strips 5 times.
7. Dispense 100 µL of Substrate Solution into the wells
8. Incubate 10–20 minutes at +18…+25 °C
9. Dispense 100 µL of Stop Solution into the wells.
10. Measure OD (optical density) at 450 nm.
11. Set photometer blank on first calibrator
Data Analysis

Calculation of Results

- Calculate the mean absorbance values (OD450) for each pair of calibrators and samples.
- Plot a calibration curve on graph paper: OD versus antigen CYFRA 21-1 concentration
- Determine the corresponding concentration of antigen CYFRA 21-1 in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.
- Below is presented a typical example of a standard curve. Not for calculations!

<table>
<thead>
<tr>
<th>Calibrators</th>
<th>Value</th>
<th>Absorbance Units (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 ng/mL</td>
<td>0.057</td>
</tr>
<tr>
<td>2</td>
<td>3 ng/mL</td>
<td>0.13</td>
</tr>
<tr>
<td>3</td>
<td>10 ng/mL</td>
<td>0.42</td>
</tr>
<tr>
<td>4</td>
<td>25 ng/mL</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>50 ng/mL</td>
<td>2.02</td>
</tr>
</tbody>
</table>
Expected Values

Each laboratory should establish its own normal range for CYFRA 21-1. The following normal range is recommended (see below). **NOTE:** the individuals that have received murine monoclonal antibodies for radioimaging or immunotreatment develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive individuals should be treated with depleting adsorbents before assaying.

<table>
<thead>
<tr>
<th>Sex, age</th>
<th>Units, ng/mL</th>
<th>Lower limit</th>
<th>Upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy donors</td>
<td>-</td>
<td>3.0</td>
<td></td>
</tr>
</tbody>
</table>

**Performance Characteristics**

Analytical specificity / Cross reactivity

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cross-reactivity, % wt/wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA125</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CA19-9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CA15-3</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>AFP</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>PSA</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Analytical sensitivity.

Sensitivity of the assay was assessed as being 0.5 ng/mL.

Precision.

- Intra-assay precision is shown below:

<table>
<thead>
<tr>
<th>Serum, no</th>
<th>duplicated</th>
<th>value, ng/mL</th>
<th>CV1, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>4.1</td>
<td>4.2</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>15.4</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>7.2</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>26.9</td>
<td>3.2</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>16.4</td>
<td>4.1</td>
</tr>
</tbody>
</table>

- Inter-assay precision is shown below:

<table>
<thead>
<tr>
<th>Serum, no</th>
<th>duplicated</th>
<th>value, ng/mL</th>
<th>CV, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>4.2</td>
<td>5.5</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>15.4</td>
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<td>3</td>
<td>5</td>
<td>7.0</td>
<td>6</td>
</tr>
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<td>4</td>
<td>5</td>
<td>24.8</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>15.9</td>
<td>4.7</td>
</tr>
</tbody>
</table>
✔ Linearity
Linearity was checked by assaying dilution series of 5 samples with different antigen CYFRA 21-1 concentrations. Linearity percentages obtained ranged within 90 to 110%.

✔ Recovery
Recovery was estimated by assaying 5 mixed samples with known antigen CYFRA 21-1 concentrations. The recovery percentages ranged from 90 to 110%.

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

References

1. Petra Stieber CYFRA 21-1 (Cytokeratin-19-Fragment), in: Lothar Thomas, Labor und Diagnose, TH Brooks, Frankfurt, Germany
Plate Layout

<table>
<thead>
<tr>
<th>12</th>
<th>11</th>
<th>10</th>
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<th>8</th>
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<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
<td>G</td>
<td>H</td>
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</table>