Human TM-CYFRA 21-1 ELISA Kit

Cat. No.:DEIA2147
Pkg.Size:96T

Intended use

The TM-CYFRA 21-1 ELISA is an enzyme immunoassay for measurement of CYFRA21-1 in serum and heparin plasma

General Description

Keratin, type I cytoskeletal 19 also known as cytokeratin-19 (CK-19) or keratin-19 (K19) is a protein that in humans is encoded by the KRT19 gene. Keratin 19 is a type I keratin. CYFRA 21-1 are its fragments.

Principle Of The Test

The TM-CYFRA 21-1 ELISA Kit 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 CYFRA21-1 molecule. An aliquot of sample containing endogenous CYFRA21-1 is incubated in the coated well with enzyme conjugate, which is an anti- CYFRA21-1 monoclonal antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of CYFRA21-1 in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of CYFRA21-1 in the sample.

Reagents And Materials Provided

1. Microtiterwells, 12x8 (break apart) strips, 96 wells; Wells coated with anti-CYFRA21-1 antibody (monoclonal)..
2. Standard (Standard 0-4), 5 vials (lyophilized), 1.0 mL; Concentrations: 0; 3; 10; 25; 50 ng/mL. Contain non-mercury preservative.
3. Control Low and High, 2 vials, (lyophilized) 1.0 mL each, Control values and ranges please refer to vial label or QC-Datasheet.Contains non-mercury preservative.
4. Sample Diluent, 1 vial, 3 mL, ready to use, Contains non-mercury preservative.
5. Assay Buffer, 1 vial, 7 mL, ready to use, Contains non-mercury preservative..
6. Enzyme Conjugate, 1 vial, 1.2 mL, ready to use, Anti-CYFRA21-1 antibody conjugated to horseradish peroxidase; Contains non-mercury preservative.
7. Substrate Solution, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).
8. Stop Solution, 1 vial, 14 mL, ready to use, contains 0.5M H_2SO_4,Avoid contact with the stop solution. It may cause skin irritations and burns.
9. Wash Solution, 1 vial, 30 mL (40X concentrated),
Note: Additional Sample Diluent for sample dilution is available upon request.

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450 ± 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

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Storage

Store all contents at 2 to 8 ℃.

Specimen Collection And Handling

Serum or heparin plasma can be used in this assay. Citrate plasma results in decreased, EDTA in strongly increased values. Do not use haemolytic, icteric or lipaemic specimens. Please note : Samples containing sodium azide should not be used in the assay.

1. Serum: Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.
2. Plasma: Whole blood should be collected into centrifuge tubes containing anti coagulant and centrifuged immediately after collection.
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 reassayed as described in Assay Steps. For the calculation of the concentrations this dilution factor has to be taken into account.

Reagent Preparation

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

1. Standards: Reconstitute the lyophilized contents of the standard vial with 1.0 mL Aqua dest. note: The reconstituted standards are stable for at least 4 weeks at 2 ℃ to 8 ℃. For longer storage freeze at -20 ℃.
2. Control: Reconstitute the lyophilized content with 1.0 mL Aqua dest. and let stand for 10 minutes in minimum. Mix the control several times before use. note: The reconstituted control is stable for at least 4 weeks at 2 ℃ to 8 ℃. For longer storage freeze at -20 ℃.
3. 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 Steps

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 50 µL of 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 diluted Wash Solution (350 µL 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 ± 10 nm with a microtiter plate reader. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

Calculation

1. Calculate the average absorbance values for each set of standards, controls and samples.

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2. Manual method: 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 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. 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

Detection Range

3 - 50 ng/mL

Sensitivity

0.15 ng/mL

Precautions

1. 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.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. The microplate contains snap-off strips. Unused wells must be stored at 2 ℃ to 8 ℃ in the sealed foil pouch and used in the frame provided.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. 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.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.

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7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (21 ℃ – 26 ℃) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. 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.
15. 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.
16. Avoid contact with Stop Solution containing 0.5 M H2SO4. It may cause skin irritation and burns.
17. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
18. 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.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

Analyte Gene Information

Gene Name	KRT19 keratin 19 [Homo sapiens]
Official Symbol	KRT19
Synonyms	KRT19; keratin 19; keratin, type I cytoskeletal 19; 40 kDa keratin intermediate filament; CK19; cytokeratin 19; K1CS; K19; keratin; type I cytoskeletal 19; type I; 40 kd; MGC15366; CK-19; keratin-19; cytokeratin-19; keratin, type I, 40-kd; 40-kDa keratin intermediate filament;
GeneID	3880
mRNA Refseq	NM_002276
Protein Refseq	NP_002267
MIM	148020
UniProt ID	P08727
Chromosome Location	17q21-q23
Pathway	Signaling mediated by p38-alpha and p38-beta, organism-specific biosystem;
Function	protein binding; structural constituent of cytoskeleton; structural constituent of muscle;

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