Human CYR61 ELISA Kit

**Cat. No.:DEIA2164**
**Pkg.Size:96T**

**Intended use**

The Cyr61 ELISA is an enzyme immunoassay for measurement of Cyr61 in serum, plasma and urine.

**General Description**

CYR61, also known as CCN1 or cysteine-rich angiogenic inducer 61, is a protein that in humans is encoded by the CYR61 gene. CYR61 is a secreted, extracellular matrix (ECM)-associated signaling protein of the CCN family (CCN intercellular signaling protein). CYR61 is capable of regulating a broad range of cellular activities, including cell adhesion, migration, proliferation, differentiation, apoptosis, and senescence through interaction with cell surface integrin receptors and heparan sulfate proteoglycans. During embryonic development, CYR61 is critical for cardiac septal morphogenesis, blood vessel formation in placenta, and vascular integrity. In adulthood CYR61 plays important roles in inflammation and tissue repair, and is associated with diseases related to chronic inflammation, including rheumatoid arthritis, atherosclerosis, diabetes-related nephropathy and retinopathy, and many different forms of cancers.

**Principle Of The Test**

The Cyr61 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 Cyr61 molecule. An aliquot of specimen sample containing endogenous Cyr61 is incubated in the coated well with assay buffer and enzyme conjugate, which is a rabbit anti-Cyr61 polyclonal antibody. After incubation, the unbound conjugate is washed off. Finally, Enzyme Complex, which is a goat anti-rabbit antibody conjugated with horseradish peroxidase, is added, and after incubation, unbound enzyme complex is washed off. The amount of bound peroxidase is proportional to the concentration of Cyr61 in the sample. Having added the substrate solution, the intensity of colour developed is proportional to the concentration of Cyr61 in the specimen sample.

**Reagents And Materials Provided**

1. Microtiterwells, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-Cyr61 antibody (monoclonal).
2. Standard (Standard 0-5), 6 vials, lyophilized, 1 mL each; Concentrations: 0; 40; 100; 250; 500; 1000 pg/mL. Contain non-mercury preservative.
3. Control Low and High, 2 vials, lyophilized, 1 mL each; See "Preparation of Reagents"; For control values and ranges please refer to vial label or QC-Datasheet. Contain non-mercury preservative.
4. Assay Buffer, 1 vial, 7 mL, ready to use, Contains non-mercury preservative.
5. Enzyme Conjugate, 1 vial, 3 mL, ready to use, rabbit anti-CYR61 antibody; Contains non-mercury preservative.
6. Enzyme Complex, 1 vial, 14 mL, ready to use, goat anti-rabbit-HRP conjugate. 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 0.5 M H2SO4. Avoid contact with the stop solution. It may cause skin irritations and burns.
9. Wash Solution, 1 vial, 30 mL (40X concentrated)

**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. Semi logarithmic graph paper or software for data reduction

**Storage**

Store all contents at 2 to 8 °C.

**Specimen Collection And Handling**

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. Urine: First clean genital area with mild desinfectant to prevent contamination. Then collect clean-catch midstream urine in an appropriate sterile container.

**Reagent Preparation**

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

1. Standard: Reconstitute the lyophilized content of the vials with 1.0 mL deionized water and let stand for 10 minutes in minimum. Mix the vials several times before use. note: The reconstituted standards are stable for 1 week at 2 °C to 8 °C.
2. Control: Reconstitute the lyophilized content of the vials with 1.0 mL deionized water and let stand for 10 minutes in minimum. Mix the vials several times before use. note: The reconstituted controls are stable for 1 week at 2 °C to 8 °C.
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**

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense 25 μL of Assay Buffer in all wells.
3. Dispense 50 μL of each Standard, Control and samples with new disposable tips into appropriate wells.
4. Dispense 25 μL Enzyme Conjugate into each well.
5. Incubate for 120 minutes at room temperature on a plate shaker with 700 rpm.
6. Briskly shake out the contents of the wells.
7. Rinse the wells 4 times with 400 μL diluted Wash Solution ( manual washing: 4 times with 300 μL diluted Wash Solution ). 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!
8. Dispense 100 μL Enzyme Complex in all wells.
9. Incubate for 30 minutes at room temperature on a plate shaker with 700 rpm.
10. Briskly shake out the contents of the wells.
11. Rinse the wells 4 times with 400 μL diluted Wash Solution ( manual washing: 4 times with 300 μL diluted Wash Solution ). Strike the wells sharply on absorbent paper to remove residual droplets.
12. Add 100 μL of Substrate Solution to each well.
13. Incubate for 15 minutes at room temperature.
14. Stop the enzymatic reaction by adding 100 μL of Stop Solution to each well.
15. Determine the absorbance (OD) of each well at 450 ± 10 nm with a microtiter plate reader.
16. 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 values and ranges 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 donor 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 donor samples.
2. Using semi-logarithmic 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 > 800 pg/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.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Optical Units (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0 (0 pg/mL)</td>
<td>0.09</td>
</tr>
<tr>
<td>Standard 1 (40 pg/mL)</td>
<td>0.17</td>
</tr>
<tr>
<td>Standard 2 (100 pg/mL)</td>
<td>0.27</td>
</tr>
<tr>
<td>Standard 3 (250 pg/mL)</td>
<td>0.57</td>
</tr>
<tr>
<td>Standard 4 (500 pg/mL)</td>
<td>1.12</td>
</tr>
<tr>
<td>Standard 5 (1000 pg/mL)</td>
<td>2.14</td>
</tr>
</tbody>
</table>

**Detection Range**
40 - 1000 pg/mL

**Sensitivity**

19.4 pg/mL

**Interferences**


**Precautions**

1. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.

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 the package insert 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 (21-26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the donor 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.

REFERENCES