CTGF (Connective Tissue Growth Factor) ELISA
For the quantitative determination of CTGF in human serum.

For Research use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 20-CTGHU-E01
Size: 96 Wells
Version: 2.0 2011/09-VK – ALPCO June 20, 2017
1 INTRODUCTION

1.1 Intended Use

The CTGF ELISA is an enzyme immunoassay for measurement of connective tissue growth factor (CTGF) in serum. For Research Use Only. Not for use in diagnostic procedures.

2 PRINCIPLE OF THE TEST

The CTGF ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a polyclonal [goat] antibody directed towards a unique antigenic site of the CTGF molecule. Together with assay buffer an aliquot of sample containing endogenous CTGF is incubated in the coated well. After incubation the unbound material is washed off. A second incubation with antiserum and a washing step follows. Enzyme conjugate, which is a goat anti-rabbit antibody conjugated with horseradish peroxidase is added. After incubation the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of CTGF in the sample thus having added the substrate solution, the intensity of color developed is proportional to the concentration of CTGF in the sample.

3 WARNINGS AND PRECAUTIONS

1. For Research 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 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 specimen 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 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 be 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 national biohazard safety guidelines or regulations.

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.

4 REAGENTS

4.1 Reagents provided

1. Microtiter wells, 12 x 8 (break apart) strips, 96 wells; Wells coated with anti-CTGF antibody (polyclonal).

2. Standard (Standard 1-5), 5 vials (lyophilized), 500 µL Concentrations: 0.10, 25.0, 50.0, 250.0, 500.0 ng/mL See “Preparation of Reagents”.

3. Control Low & High, 2 vials, (lyophilized), 500 µL see “Reagent Preparation” For control values and ranges please refer to vial label or QC-Datasheet.

4. Assay Buffer, 1 vial, 20 mL, ready to use, Contains non-mercury preservative.

5. Antiserum, 1 vial, 14 mL, ready to use, Rabbit anti-CTGF antibody Contains non-mercury preservative.

6. Enzyme Conjugate, 1 vial, 14 mL, ready to use, Anti-rabbit 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 H₂SO₄ Avoid contact with the stop solution. It may cause skin irritations and burns.

9. Wash Solution, 1 vial, 30 mL (40X concentrated), see “Preparation of Reagents”.

Note: Additional Assay Buffer for sample dilution is available upon request.

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 ± 10 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled or deionized water
- Timer
- Graph paper or software for data reduction

4.3 Storage Conditions

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.

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

**Standard**
Reconstitute the lyophilized contents of the standard vials with 500 µL Assay Buffer and let stand for 10 minutes at minimum. Mix the control several times before use.

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

**Control**
Reconstitute the lyophilized content of the control vials with 500 µL Assay Buffer and let stand for 10 minutes at minimum. Mix the control several times before use.

*Note: The reconstituted controls are stable for 2 days 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.*

4.5 Disposal of the Kit
The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits
In case of any severe damage to the test kit or components, ALPCO has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.
5 SAMPLE COLLECTION AND PREPARATION

Serum can be used in this assay.
Do not use hemolyzed, icteric or lipemic samples.
Hemolyzed samples result in strongly decreased values!
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Sample Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), 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.

Sample Storage and Preparation
Samples should be capped and may be stored for up to 3 days at 2 °C to 8 °C prior to assaying. Samples held for a longer time (up to six months) should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.2 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with Assay Buffer 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 Serum + 90 µL Assay Buffer (mix thoroughly)
b) dilution 1:100: 10 µL dilution a) 1:10 + 90 µL Assay Buffer (mix thoroughly)

6 ASSAY PROCEDURE

6.1 General Remarks
- All reagents and samples must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposable plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- 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.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
6.2 Test Procedure

Each run must include a standard curve.

1. Secure the desired number of microtiter wells in the frame holder.
2. Dispense 150 µL Assay Buffer into each well.
3. Add 25 µL of each Standard, Control and sample with new disposable tips into appropriate wells.
   Thoroughly mix for 10 seconds. It is important to have complete mixing in this step.
4. Incubate for 120 minutes at room temperature.
5. 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!
6. Dispense 100 µL Antiserum into each well.
7. Incubate for 60 minutes at room temperature.
8. 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.
9. Add 100 µL of Enzyme Conjugate to each well.
10. Incubate for 30 minutes at room temperature.
11. 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.
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. It is recommended that the wells be read within 10 minutes after adding the Stop Solution.

6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and samples from each standard.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with the 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 >500 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.
6.3.1 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.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Optical Units (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1 (0.10 ng/mL)</td>
<td>0.16</td>
</tr>
<tr>
<td>Standard 2 (25 ng/mL)</td>
<td>0.28</td>
</tr>
<tr>
<td>Standard 3 (50 ng/mL)</td>
<td>0.42</td>
</tr>
<tr>
<td>Standard 4 (250 ng/mL)</td>
<td>1.29</td>
</tr>
<tr>
<td>Standard 5 (500 ng/mL)</td>
<td>2.09</td>
</tr>
</tbody>
</table>

7 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.

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.

Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of the control materials, assay 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.

After checking the above mentioned items without finding any error contact your distributor or ALPCO directly.

8 PERFORMANCE CHARACTERISTICS

8.1 Assay Dynamic Range

The range of the assay is between 3.2 – 500 ng/mL.

8.2 Specificity of Antibodies (Cross-Reactivity)

The following substances were tested for cross-reactivity of the assay:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration up to</th>
<th>Cross-reactivity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOV</td>
<td>32 ng/mL</td>
<td>0.0</td>
</tr>
<tr>
<td>CYR61</td>
<td>1 ng/mL</td>
<td>0.1</td>
</tr>
<tr>
<td>WISP</td>
<td>130,000 ng/mL</td>
<td>0.0</td>
</tr>
<tr>
<td>WISP</td>
<td>200,000 ng/mL</td>
<td>0.0</td>
</tr>
<tr>
<td>WISP</td>
<td>255,000 ng/mL</td>
<td>0.0</td>
</tr>
</tbody>
</table>
8.3 Sensitivity
The analytical sensitivity of the CTGF ELISA Kit was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Standard 1 and was found to be 3.2 ng/mL.

8.4 Reproducibility
8.4.1 Intra-Assay
The within assay variability is shown below:

<table>
<thead>
<tr>
<th>Samples</th>
<th>n</th>
<th>Mean (ng/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>110.1</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>49.9</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>129.7</td>
<td>3.9</td>
</tr>
</tbody>
</table>

8.4.2 Inter-Assay
The between assay variability is shown below:

<table>
<thead>
<tr>
<th>Samples</th>
<th>n</th>
<th>Mean (ng/mL)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>27.2</td>
<td>9.7</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>15.0</td>
<td>9.8</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>31.0</td>
<td>10.6</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>32.2</td>
<td>6.2</td>
</tr>
</tbody>
</table>

8.5 Recovery

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>179.2</td>
<td>494.6</td>
</tr>
<tr>
<td>Average Recovery</td>
<td>101.9</td>
<td>89.2</td>
</tr>
<tr>
<td>Range of Recovery [%] from to</td>
<td>95.1 114.3</td>
<td>86.2 97.9</td>
</tr>
</tbody>
</table>

8.6 Linearity

<table>
<thead>
<tr>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>179.2</td>
<td>140.0</td>
</tr>
<tr>
<td>Average Recovery</td>
<td>102.7</td>
<td>102.6</td>
</tr>
<tr>
<td>Range of Recovery [%] from to</td>
<td>93.3 113.8</td>
<td>100.6 106.3</td>
</tr>
</tbody>
</table>

9 LIMITATIONS OF USE
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.
9.1 Interfering Substances
Billirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results. Hemolyzed samples result in strongly decreased values.

9.2 Drug Interferences
No substances (drugs) are currently known to us that have an influence on the measurement of CTGF in a sample.

10 LEGAL ASPECTS
10.1 Reliability 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 national 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. In case of any doubt or concern please contact ALPCO.

10.2 Liability
Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

11 REFERENCES / LITERATURE