Human CTGF ELISA Kit

Cat #: orb1149602 (manual)

For the quantitative in vitro determination of Human connective tissue growth factor concentrations in serum - plasma - tissue homogenates - other biological fluids.

For research use only. Not for use in diagnostic procedures. This package insert must be read in its entirety before using this product.

INTENDED USE AND TEST PRINCIPLE

This CTGF；CCN2 ELISA kit is intended Laboratory for Research use only and is not for use in diagnostic or therapeutic procedures. The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of CTGF；CCN2 in the sample, this CTGF；CCN2 ELISA Kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of Optical Density versus CTGF；CCN2 concentration. The concentration of CTGF；CCN2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

SAMPLE COLLECTION AND STORAGES

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4℃ before centrifugation for 20 minutes at approximately 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8℃ within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS (the volume depends on the weight of the tissue. 9mL PBS would be appropriate to 1 gram tissue pieces. Some

protease inhibitor is recommended to add into the PBS.) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Cell culture supernates and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

Note: The samples should be centrifugated adequately and no hemolysis or granule was allowed.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 ℃ incubator
2. Standard microplate reader capable of measuring absorbance at 450 nm
3. Precision pipettes, disposable pipette tips and Absorbent paper
4. Distilled or deionized water

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

Name	96 determinations	48 determinations
MICROTITER PLATE	12*8strips	12*4strips
STANDARD（6 vial）	0.3ml/vial	0.3ml/vial
SAMPLE DILUENT	6.0ml	3.0ml
ENZYME CONJUGATE	10.0ml	5.0ml
WASH SOLUTION	25ml	15ml
SUBSTRATE A	6.0ml	3.0ml
SUBSTRATE B	6.0ml	3.0ml
STOP SOLUTION	6.0ml	3.0ml
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1

Note:

1. Standard concentration was followed by: 2000, 1000, 500, 250, 125, 62.5 pg/mL.
2. If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate viruses.
10. Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
11. Substrate Solution is easily contaminated. If bluish prior to use, do not use.
12. Substrate B contain 20% acetone, keep this reagent away from sources of heat or flame.
13. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

REAGENT PREPARATION AND STORAGE

Wash Solution (1X) - Dilute 1 volume of Wash solution (20X) with 19 volumes of deionized or distilled water. Wash Solution is stable for 1 month at 2-8°C.

ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microtiter plate.
2. Add 50µl of Standard or Sample to the appropriate wells. Blank well doesn't add anything.
3. Add 100µl of Enzyme conjugate to standard wells and sample wells except the blank well, cover with an adhesive strip and incubate for 60 minutes at 37°C.
4. Wash the Microtiter Plate 4 times.

Manual Washing - Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Solution (1X), then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure for a total of four times. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing - Aspirate all wells, then wash plates four times using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350µL/well/wash. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

5. Add Substrate A 50µl and Substrate B 50µl to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**
6. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

CALCULATION OF RESULTS

1. This standard curve is used to determine the amount in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (X) axis versus the corresponding concentration on the horizontal (Y) axis.

2. First, calculate the mean O.D. value for each standard and sample. All O.D. Values are subtracted by the mean value of the blank well before result interpretation. Construct the standard curve using graph paper or statistical software.

3. To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.

4. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

5. Intra-assay CV (%) is less than 10% and Inter-assay CV (%) is less than 15%.

6. Assay range: 62.5 pg/mL – 2000 pg/mL.

7. Sensitivity: The minimum detectable dose of Human CTGF； CCN2 is typically less than 10 pg/mL.

8. Cross-reactivity: This assay recognizes recombinant and natural Human CTGF； CCN2. No significant cross-reactivity or interference was observed.

9. Storage: 2-8℃ (Use frequently) ; six months (-20℃) 。

10. Standard curve