HUMAN CONNECTIVE TISSUE GROWTH FACTOR (CTGF) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CTGF CONCENTRATIONS IN CELL CULTURE SUPERNATES, SERUM, AND PLASMA.

PURCHASE INFORMATION:

ELISA NAME	HUMAN CTGF ELISA
Catalog No.	SK00726-02
Formulation	96 T
Standard range	31-2000 ng/ml
Sensitivity	21 ng/ml
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma, cell culture
Specificity	Human CTGF (38KD) and C-Terminal fragments (16-20KD)
Intra-assay Precision	6-8%
Inter-assay Precision	10-12%
Storage	4 °C

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Human CTGF Immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human CTGF in cell culture supernates, serum, and plasma. It contains recombinant human CTGF and antibodies raised against this protein. It has been shown to accurately quantitie recombinant human CTGF. Results obtained with naturally occurring CTGF samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the Immunoassay kit can be used to determine relative mass values for natural human CTGF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for CTGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CTGF present is bound by the immobilized antibody. After washing away any unbound substances, a polyclonal antibody specific for CTGF is added to the wells. Following a wash to remove any unbound antibody, HRP link Anti Goat IgG is add to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of CTGF bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
CTGF Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with antibody against human CTGF.	726-02-01	1 plate
CTGF Standard – 2 µg/vial of recombinant human CTGF in a buffered protein base with preservatives; lyophilized.	726-02-02	1 vial
Detection Antibody Concentrate– 120 µL / vial, 100-fold concentrated of polyclonal antibody against human CTGF with preservatives; lyophilized.	726-02-03	1 vial
Positive Control- one of recombinant human CTGF, lyophilized	726-02-04	1 vial
Anti Goat IgG-HRP Conjugate -120 ul/vial, 100-fold concentrated solution of Anti Goat IgG conjugate to HRP	AGIGHRP	1 vial
Dilution Buffer- 60mL/vial of buffered protein based solution with preservatives	DB01	1 vial
HRP Dilute Solution-12 mL/vial of buffered protein based solution with preservatives	DB06	1 vial
Wash Buffer -50 ml/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution-11 ml / vial of TMB substrate solution	TMB01	1 vial
Stop Solution- 11 ml /vial of 0.5 M HCL solution	S-STOP	1 vial
Plate Covers – Plate sealer.	EAPS	1

STORAGE

Unopened Kit: Store at 2 - 8° C. Do not use past kit expiration date.

Opened / Reconstituted Reagents: May be stored for up to 1 month at 2 - 8°C.
Standard should be stored for up to 1 month at -70° C.
Microplate Wells: Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

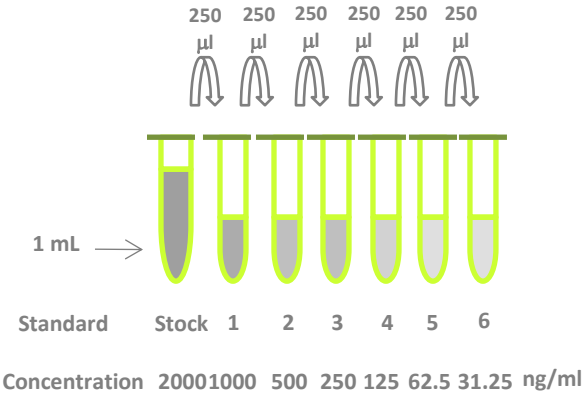
SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.
CTGF Standard - Refer to vial label for reconstitution volume. Reconstitute the **CTGF** Standard with 1 ml of Dilution Buffer. This reconstitution produces a stock solution of 2000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tube #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 ng/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 ml	2000 ng/ml
# 1	250µl of stock	250µl	1000 ng/ml
# 2	250µl of 1	250µl	500 ng/ml
# 3	250µl of 2	250µl	250 ng/ml
# 4	250µl of 3	250µl	125 ng/ml
# 5	250µl of 4	250µl	62.5 ng/ml
# 6	250µl of 5	250µl	31.25 ng/ml



Detection Antibody- Reconstitute the **Detection Antibody concentrated** with 120 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 11. 88 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution.

Anti Goat IgG-HRP Conjugate - Pipette 11. 88 mL of HRP Dilute Solution into the 15 ml centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. *Note: 1 x working solution of Anti Goat IgG-HRP Conjugate should be used within a few days.*

Positive Control- Reconstitute the **Positive Control** with 1.0 mL of Dilution Buffer. *Positive Control should be prepared and used immediately*.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (B2, B3).
4. Add 100 µL of Standard (from C2 to G3, G4 to F5), sample, or control per well (E4, E5). Cover with the Sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **Anti Goat IgG-HRP Conjugate** working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 15-20 minutes at room temperature. **Protect from light.**
11. Add 100 µL of 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 if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a Log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the CTGF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

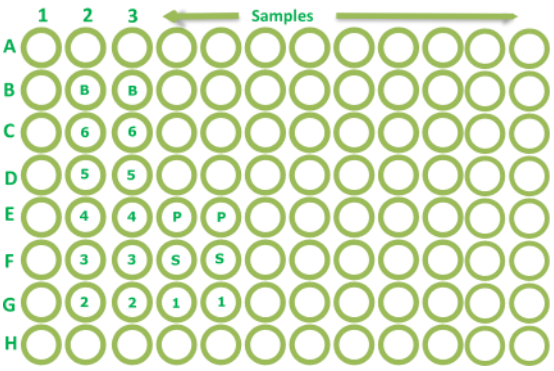
STANDARD (NG/ML)	AVERAGE OD450 (CORRECTED)
31.25	0.010
62.5	0.022
125	0.066
250	0.158
500	0.335
1000	0.724
2000	1.566

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human CTGF.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of CTGF Was 21 ng/mL.



SPECIFICITY

This assay recognizes both natural and recombinant human CTGF. Both antibodies recognize full length (38KD) and C-terminal fragments (18-20KD) on western blot analysis. The factors listed below were prepared at 50µg/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

PROTEIN	CROSSREACTIVITY (%)
Human CTGF	100
Human SPARC	0
Human CTGFL/WISP2	0
Human TGF-β1	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Anti Goat IgG HRP conjugate to each well. Incubate 1 hour on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate to each well. Incubate 15-20 min on the bench top. Protect from light.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min