HUMAN CONNECTIVE TISSUE GROWTH FACTOR (CTGF) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN CTGF CONCENTRATIONS IN SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN CTGF ELISA
Catalog No.	SK00726-01
Lot No.	
Formulation	96 T
Standard range	62.5-4000 pg/mL
Sensitivity	31 pg/mL
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma
Dilution factor	Optimal dilutions should be determined by each laboratory for each application
Specificity	Human CTGF
Intra-assay Precision	4-6%
Inter-assay Precision	8-10%
Storage	2-8°C

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INTRODUCTION

Human CTGF immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human CTGF in serum and plasma. It contains recombinant human CTGF and antibodies raised against this protein. It has been shown to accurately quantify 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 biotinylated polyclonal antibody specific for CTGF is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Avidin is added 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.
_ Centrifuge vials prior to opening.
_ 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 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 an antibody against CTGF.	**726-01-01**	**1 plate**
CTGF Standard – 4000 pg/vial of recombinant human CTGF in a buffered protein base with preservatives; lyophilized.	**726-01-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of biotinylated polyclonal antibody against CTGF with preservatives; lyophilized.	**726-01-03**	**1 vial**
Positive Control - one vial of recombinant human CTGF, lyophilized	**726-01-04**	**1 vial**
Avidin-HRP Conjugate – 120 µl/vial, 100-fold concentrated solution of Avidin conjugate to HRP	**AVHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservatives	**DB07**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
ABTS Substrate Solution - 11 mL ABTS substrate solution	**ABTS01**	**1 bottle**
Stop Solution - 11 mL of 0.9% SDS solution	**SDS-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard and Detection Antibody Concentrate Solution SHOULD BE STORED at -20 °C or -70°C for up to one month. Avidin-HRP Conjugate 100-fold

concentrated and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 405nm or 650nm.
- 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

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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Samples do not require dilution, but optimal dilutions should be determined by each laboratory for each application with a sample pretest.
Use polypropylene test tubes.

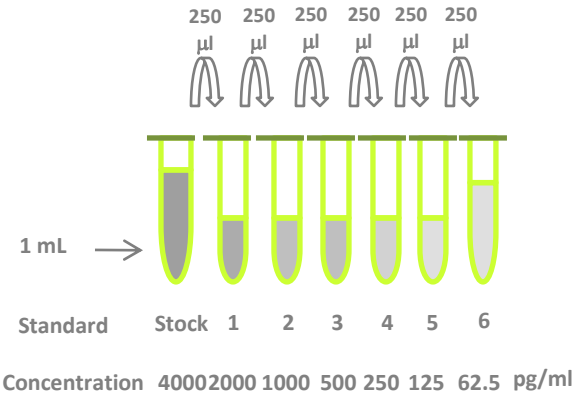
REAGENT PREPARATION

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 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 mL	4000 pg/ml
# 1	250µl of stock	250µl	2000 pg/ml
# 2	250µl of 1	250µl	1000 pg/ml
# 3	250µl of 2	250µl	500 pg/ml
# 4	250µl of 3	250µl	250 pg/ml
# 5	250µl of 4	250µl	125 pg/ml
# 6	250µl of 5	250µl	62.5 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody concentrate with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of Dilution Buffer into a 15 ml centrifuge tube and transfer 105 µl of 100-fold concentrated stock solution to prepare working solution.

Avidin-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of

Avidin-HRP Conjugate should be used within a few days.

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

ASSAY PROCEDURE

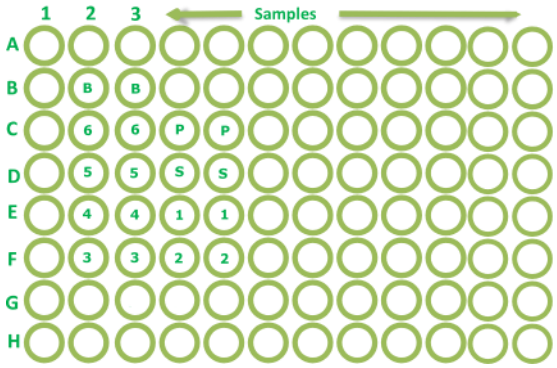
Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

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 plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard (from C2, C3 to F2, F3, and D4, D5 to F4, F5), sample, or positive control (C4, C5) per well. Cover with plate 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 1x 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 Avidin-HRP Conjugate working solution to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 20-30 minutes at room temperature. **Protect from light.**
11. That yields a green end product upon reaction with peroxidase. The green product has two major absorbance peaks, 405 nm and 650 nm. Add 100 µL of Stop Solution to each well.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 405 nm or 650 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive 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.



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 31 pg/mL.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD405 (CORRECTED)
Blank	0 (0.160)
62.5	0.040
125	0.059
250	0.096
500	0.102
1000	0.183
2000	0.340
4000	0.683

- **Lot No.:**
- **** Positive Control: 100 - 500 pg/mL**

SPECIFICITY

This assay recognizes both natural and recombinant human CTGF. The factors listed below were prepared at 50 ng/mL in Dilution Buffer, and assayed for cross reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rh CTGF control were assayed for interference. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY
Human CTGF	100%
Human CTGF, Full Length, His Tag	100%
WISP-2	0
WISP-3	0
BMP4	0
BMP-5	0
BMP-9	0
DKK1	0
TGF-β1	0
NTF3	0
SPARC	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl 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 working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Avidin-HRP conjugate working solution to each well. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 20-30 min on the plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 405nm or 650nm within 15 min