HUMAN CONNECTIVE TISSUE GROWTH FACTOR (CTGF) ELISA KIT

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

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN CTGF ELISA
Catalog No.	SK00726-03
Lot No.	
Formulation	96 T
Standard range	2-128 ng/ml
Sensitivity	500 pg/ml
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma
Sample Dilution	*Optimal dilutions should be determined by each laboratory for each application*
Specificity	Human CTGF (38KD) and C-Terminal Fragments (16-20 KD)
Intra-assay Precision	6 - 8%
Inter-assay Precision	8 - 12%
Storage	2 – 8 °C

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INTRODUCTION

Human CTGF immunoassay is a 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 antibody specific for CTGF is added to the wells. Following a wash to remove any unbound antibody, HRP link Streptavidin 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

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_ 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 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.	**726-03-01**	**1 plate**
CTGF Standard – 64 ng/vial of recombinant human CTGF in a buffered protein base with preservative; lyophilized.	**726-03-02**	**2 vials**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of an antibody against CTGF with preservative; lyophilized.	**726-03-03**	**1 vial**
Positive Control - one vial of recombinant human CTGF; lyophilized.	**726-03-04**	**1 vial**
Streptavidin -HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB18**	**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 piece**
Plastic Pouch	**P01**	**1 piece**

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 (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C or -70 °C for up to one month. SAHRP Conjugate

100-fold concentrated solution (protect from light) and other components may be stored at 2 – 8 °C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch (P01) with desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 405 nm or 650 nm.
- Microplate shaker (250-300 rpm).
- 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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

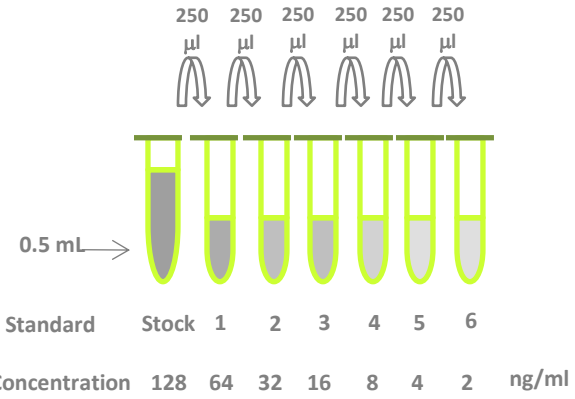
SAMPLE PREPARATION

Plasma and Serum samples may not require dilution. **Optimal dilutions should be determined by each laboratory for each application with a 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 1x Wash Buffer.
CTGF Standard - Refer to vial label for reconstitution volume. Reconstitute the **CTGF** standard with 0.5 mL of Dilution Buffer. This reconstitution produces a stock solution of 128 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 tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 128 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	0.5 ml	128 ng/ml
# 1	250µl of stock	250µl	64 ng/ml
# 2	250µl of 1	250µl	32 ng/ml
# 3	250µl of 2	250µl	16 ng/ml
# 4	250µl of 3	250µl	8 ng/ml
# 5	250µl of 4	250µl	4 ng/ml
# 6	250µl of 5	250µl	2 ng/ml



Detection Antibody - Reconstitute the **Detection Antibody Concentrate** with 1.05 mL of **Antibody Diluent Solution (DB18)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody Diluent Solution into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-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 Streptavidin-HRP Conjugate should be used within a few days (protect from light).*

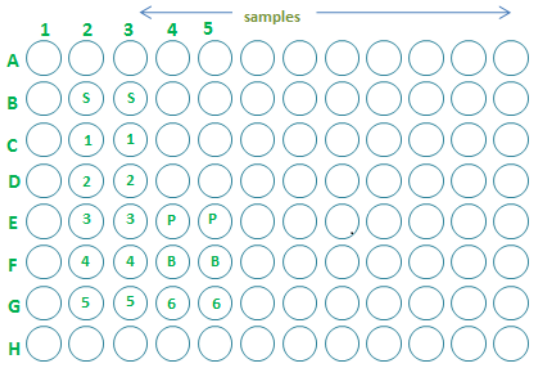
Positive Control - Reconstitute the **Positive Control** with 0.5 mL of Dilution Buffer. *Note: Positive Control*

could be reused within a few days if stored at -20 °C or -70 °C.

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 duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells (F4, F5).
4. Add 100 µL of Standard solutions in reverse order of serial dilution (G4, G5 and G2, G3 to B2, B3), sample, or positive control (E4, E5) per well. Cover with plate sealer. Incubate for 2 hours on microplate 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 plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution to each well. Incubate for 1 hour on microplate 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 7-15 minutes on microplate shaker at room temperature. **Protect from light.**
11. This 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 microplate 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

The minimum detectable dose (MDD) of CTGF was 500 pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human CTGF. No significant cross-reactivity or interference was observed.

HUMAN :
BMP-4, CTGFL/WISP-2, BMP5, BMP9, DKK1, NTF3, TGF-β, WISP-1, WISP-3

TYPICAL DATA

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

STANDARD (NG/ML)	AVERAGE OD405NM (CORRECTED)
Blank	0 (0.151)
2	0.004
4	0.017
8	0.071
16	0.148
32	0.275
64	0.479
128	0.748

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control to the 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 SAHRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 7-15 minutes on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 405 nm or 650 nm within 15 minutes.