

HUMAN CONNECTIVE TISSUE GROWTH FACTOR (CTGF) ELISA KIT

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



ALWAYS REFER TO LOT SPECIFIC PROTOCOL
PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ BEFORE USING THIS PRODUCT.

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

PURCHASE INFORMATION:

ELISA NAME	HUMAN CTGF ELISA
Catalog No.	SK00726-01
Lot No.	
Formulation	96 T
Standard range	125 - 8000 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
Calibration	Human CTGF recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This Human CTGF ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human CTGF from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human CTGF and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural CTGF samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human CTGF. The capture antibody can bind to the human CTGF in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human CTGF is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (ABTS) is added to the wells and color develops in direct proportion to the amount of human CTGF bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

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

_This ELISA kit should not be used beyond the expiration date on the kit label.

_Do not mix 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.

_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

Description	Code	Quantity
CTGF Microplate - 96 well microplate coated with an antibody specific for human CTGF.	726-01-01	1 plate
CTGF Standard – 8000 pg/vial of lyophilized recombinant human CTGF.	726-01-02	1 vial
Detection Antibody Concentrate – 1.05 mL/vial of 10-fold concentrate of lyophilized biotinylated antibody against human CTGF.	726-01-03	1 vial
Positive Control - one vial of lyophilized recombinant human CTGF.	726-01-04	1 vial
Streptavidin-HRP Conjugate – 120 µL/vial of 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.	DB08	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 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C 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. Streptavidin-HRP 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 with the 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 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.

Other sample types, such as cell culture supernatants, need to be validated prior to assay. For cell cultures supernates, must use **animal serum free media**. Fetal bovine serum samples cross-reacts with this ELISA Kit.

Optional: Use Aprotinin (enzyme inhibitor) 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

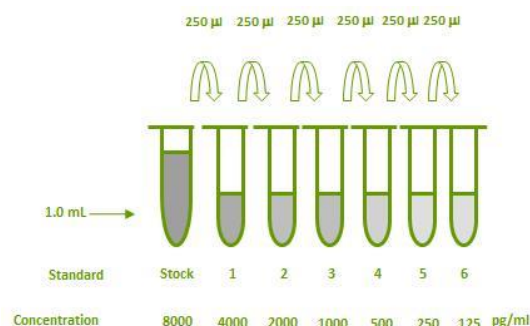
Bring all reagents to room temperature before use.

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 1x Wash Buffer.

CTGF Standard - Reconstitute the CTGF standard with 1.0 mL of **Dilution Buffer (DB06)**. This reconstitution produces a stock solution of 8000 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 **8000 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	8000 pg/ml
# 1	250µl of stock	250µl	4000 pg/ml
# 2	250µl of 1	250µl	2000 pg/ml
# 3	250µl of 2	250µl	1000 pg/ml
# 4	250µl of 3	250µl	500 pg/ml
# 5	250µl of 4	250µl	250 pg/ml
# 6	250µl of 5	250µl	125 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of **Dilution Buffer (DB06)**. **Note:** Positive control could be reused within a few days if stored at -20° C or -70° C.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of **Antibody Diluent Solution (DB08)** to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Antibody Diluent Solution (DB08) 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 (DB06)** 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**).

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

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 per well of Dilution Buffer to Blank wells.
4. Add 100 µL of Standard dilutions, sample, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
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 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of ABTS Substrate Solution to each well. Incubate for 25-35 minutes on microplate shaker at room temperature. **Protect from light.**
11. This will yield 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 SDS Stop Solution to each well.

12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 405nm or 650nm.

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.

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 NM (CORRECTED)
Blank	0 (0.099)
125	0.026
250	0.059
500	0.120
1000	0.262
2000	0.463
4000	0.755
8000	1.099

- **Lot No.:**
- **Positive Control:**

SPECIFICITY

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- β 1	0
NTF3	0
SPARC	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 100 μ l of standard dilutions, samples, or positive control to each well. Incubate 2 hours on the plate shaker at RT.

Aspirate and wash 4 times.

Add 100 μ l Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.

Aspirate and wash 4 times.

Add 100 μ l Streptavidin-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 μ l ABTS Substrate Solution to each well. Incubate 25-35 min on the plate shaker at RT. Protect from light.

Add 100 μ l Stop Solution to each well. Read 405nm or 650nm within 15 min.