HUMAN CONNECTIVE TISSUE GROWTH FACTOR (CTGF) ELISA KIT

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

PURCHASE INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN CTGF ELISA
Catalog No.	SK00726-06
Lot No.	
Formulation	96 T
Standard range	62.5 - 4000 pg/mL
Sensitivity	30 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 approximately 40 samples duplicated provided that assay is run according to protocol.	

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

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

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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.
_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 – lot specific of lyophilized recombinant human CTGF.	**726-01-02**	**1 vial**
Detection Antibody Concentrate – lot specific 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 – 30 mL of buffered protein based solution with preservative.	**DB16**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB68C**	**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 10 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.

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.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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 lot specific of Dilution Buffer. 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 #2 to #7. 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	Lot specific	
# 1	Lot specific	Lot specific	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
# 7	250µl of 6	250µl	62.5 pg/ml

Positive Control - Reconstitute the Positive Control with lot specific of Dilution Buffer.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with lot specific of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of Dilution Buffer 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 **HRP Diluent Solution (DB68C)** into a 15 mL centrifuge tube and transfer 120 µl of 100-fold concentrated stock solution to prepare working solution **(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. Add 100 µL per well of Dilution Buffer to Blank wells.
3. Add 100 µL of Standard dilutions, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. 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.
5. 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.
6. Repeat the aspiration/wash as in step 4.
7. 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.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of ABTS Substrate Solution to each well. Incubate for 27-30 minutes on microplate shaker at room temperature. **Protect from light.**
10. This will yield a green end product upon reaction with peroxidase. The green product has two major absorbance peaks, 405nm and 650nm. Add 100 µL of SDS Stop Solution to each well.
11. Determine the optical density of each well using a microplate reader set to 405nm.

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 (0.140)
62.5	0.039
125	0.083
250	0.173
500	0.334
1000	0.520
2000	0.880
4000	1.661

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-β1	0
NTF3	0
SPARC	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS

Add 100 µl 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 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 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 µl ABTS Substrate Solution to each well. Incubate 27-30 min on the plate shaker at RT. **Protect from light**.

Add 100 µl Stop Solution to each well. Read at 405nm.