MANUAL

CTGF KITS

Part # 64CTGFPEG & 64CTGFPEH

Test size#: 500 tests (64CTGFPEG) and 10,000 tests (64CTGFPEH) - assay volume: 20 µL

Revision: #02 of September 2023

Store at: -60°C or below (64CTGFPEG); -60°C or below (64CTGFPEH)

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Human CTGF in cell/tissue culture supernatants and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, Human CTGF is detected in a sandwich assay by using anti CTGF antibody labeled with Europium cryptate (donor), and anti CTGF antibody labeled with d2 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the Human CTGF concentration.

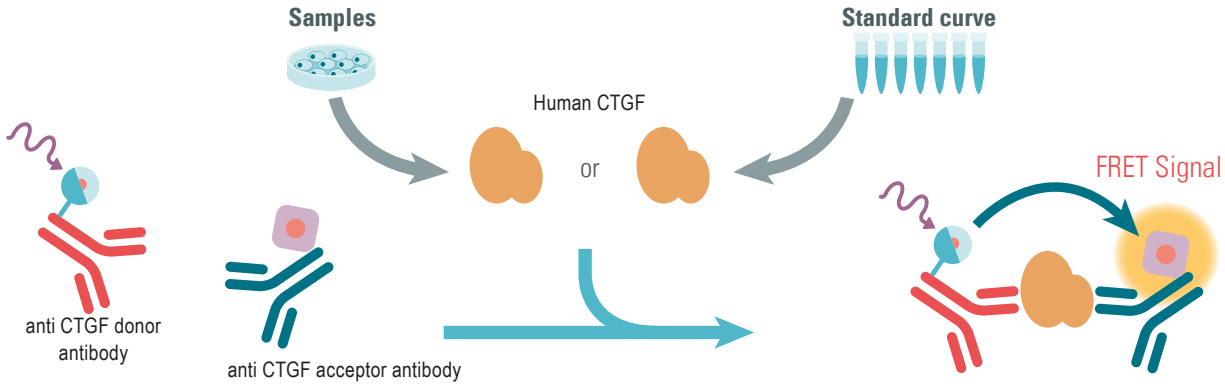
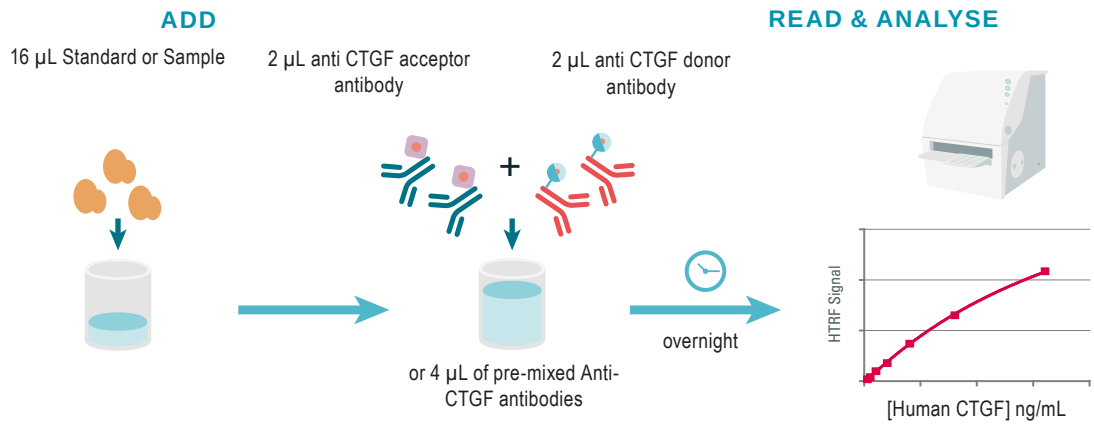


Figure 1: Principle of HTRF Human CTGF sandwich assay.

MANUAL AT A GLANCE



Make sure to use the set-up for Eu Cryptate.

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 64CTGFPEG	10,000 TESTS * CAT # 64CTGFPEH
Human CTGF Standard Frozen	1 vial - 50 µL 4750 ng/mL	1 vial - 50 µL 4750 ng/mL
Human CTGF Eu Cryptate Antibody	1 vial - 20 µL Frozen - 50X	1 vial - 0.4 mL Frozen - 50X
Human CTGF d2 Antibody	1 vial - 20 µL Frozen - 50X	1 vial - 0.4 mL Frozen - 50X
Diluent #5 ** 5X	1 vial 2 mL	1 vial 10 mL
Detection buffer *** ready-to-use	2 vials 1.5 mL Detection Buffer #3	1 vial 50 mL Detection Buffer #3

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume..
Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
** Medium like cell culture medium can be an alternative to the diluent.
*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

PURCHASE SEPARATELY:

- HTRF®-Certified Reader. Make sure the setup for Eu Cryptate is used.

For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvity.com

- Small volume (SV) detection microplates - Use white plate only.

For more information about microplate recommendations, please visit our website at: www.revvity.com

STORAGE AND STABILITY

Store the kit at -60°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label. Diluent and detection buffer are shipped frozen, but can be stored at 2-8°C in your premises.

If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below .

Volume of CTGF standard aliquots should not be under 10 µL.

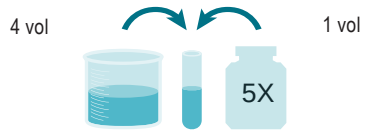
REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature, allow them to warm up to room temperature for at least 30 mins before use
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- Human CTGF standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

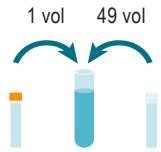
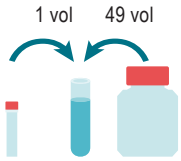
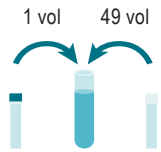
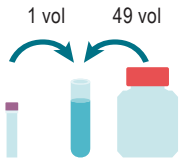
TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64CTGFPEG		10,000 TESTS KIT - 64CTGFPEH
Anti-Human CTGF Eu Cryptate antibody		
Thaw the Human CTGF Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.		Thaw the Human CTGF Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.
Anti-Human CTGF d2 antibody		
Thaw the Human CTGF d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.		Thaw the Human CTGF d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.
Human CTGF Standard		
Thaw the CTGF standard solution in order to obtain a 4750 ng/mL stock solution. Mix gently.		Thaw the CTGF standard solution in order to obtain a 4750 ng/mL stock solution. Mix gently.
Diluent		
Dilute 5-fold the 5 X diluent #5 with distilled water: homogenize the 5 X diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water (e.g., 1 mL of diluent + 4 mL of distilled water). Mix gently after dilution. This 1X diluent can be frozen and stored at -60°C or below.		Dilute 5-fold the 5 X diluent #5 with distilled water: homogenize the 5 X diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water (e.g., 10 mL of diluent + 40 mL of distilled water). Mix gently after dilution. This 1X diluent can be frozen and stored at -60°C or below.
Detection buffer		
The Detection buffer is ready-to-use.		The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 2 µL of Human CTGF-Eu Cryptate Antibody and 2 µL of Human CTGF-d2 Antibody.

Prepare the two antibody solutions in separate vials.

500 TESTS KIT - 64CTGFPEG		10,000 TESTS KIT - 64CTGFPEH
Human CTGF Eu Cryptate antibody		
Dilute 50-fold the 50X stock solution (thawed reagent) of CTGF Eu Cryptate antibody stock solution with the Detection buffer #3 : add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 20 µL of Eu Cryptate antibody stock solution + 980 µL of Detection Buffer #3).	 	Dilute 50-fold the 50X stock solution (thawed reagent) of CTGF Eu Cryptate antibody stock solution with the Detection buffer #3 : add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 0.4 mL of Eu Cryptate antibody stock solution + 19.6 mL of Detection Buffer #3).
Human CTGF d2 antibody		
Dilute 50-fold the 50X stock solution (thawed reagent) of CTGF d2 antibody stock solution with the Detection buffer #3 : add 1 volume of d2-antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 20 µL of d2-antibody stock solution + 980 µL of Detection Buffer #3).	 	Dilute 50-fold the 50X stock solution (thawed reagent) of CTGF d2 antibody stock solution with the Detection buffer #3 : add 1 volume of d2 antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 0.4 mL of d2 antibody stock solution + 19.6 mL of Detection Buffer #3).
Antibody mix		
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).		It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 16 µL of standard.
- Dilute the standard stock solution serially with diluent #5 (1X)
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent #5 (1X).
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

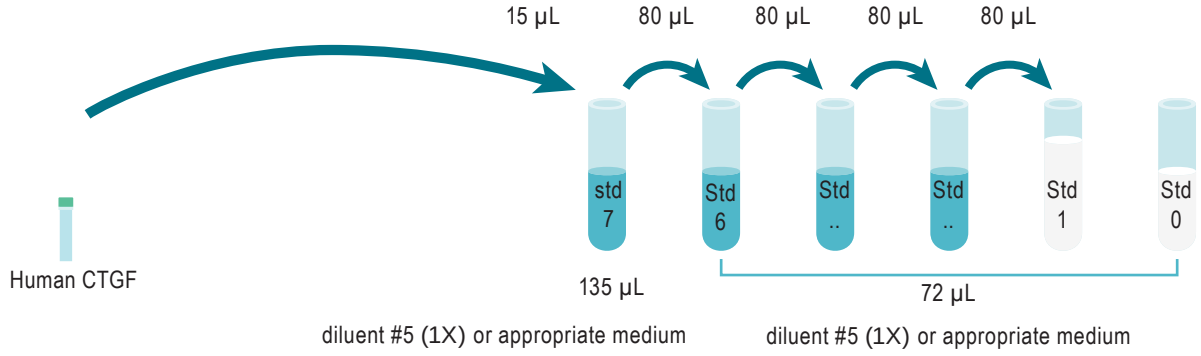
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 10-fold with diluent #5 (1X) to prepare high standard (std 7): e.g. take 15 µL of standard stock solution and add it to 135 µL of diluent #5 (1X). Mix gently.

Use the high standard (std 7) to prepare the standard curve using 1/1.9 serial dilutions as follows:

- Dispense 72 µL of diluent #5 (1X) in each vial from Std 6 to Std 0.
- Add 80 µL of standard to 72 µL of diluent #5 (1X), mix gently and repeat the 1/1.9 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Negative control) is diluent #5 (1X) or appropriate culture medium alone.



STANDARD	SERIAL DILUTIONS	CTGF WORKING SOLUTIONS (NG/ML)
Standard Stock solution	Thawed stock solution	4750
Standard 7	15 µL stock solution + 135 µL Diluent #5 (1X)	475
Standard 6	80 µL standard 7 + 72 µL Diluent #5 (1X)	250
Standard 5	80 µL standard 6 + 72 µL Diluent #5 (1X)	131.60
Standard 4	80 µL standard 5 + 72 µL Diluent #5 (1X)	69.30
Standard 3	80 µL standard 4 + 72 µL Diluent #5 (1X)	36.45
Standard 2	80 µL standard 3 + 72 µL Diluent #5 (1X)	19.20
Standard 1	80 µL standard 2 + 72 µL Diluent #5 (1X)	10.10
Standard 0	100 µL Diluent #5 (1X)	0

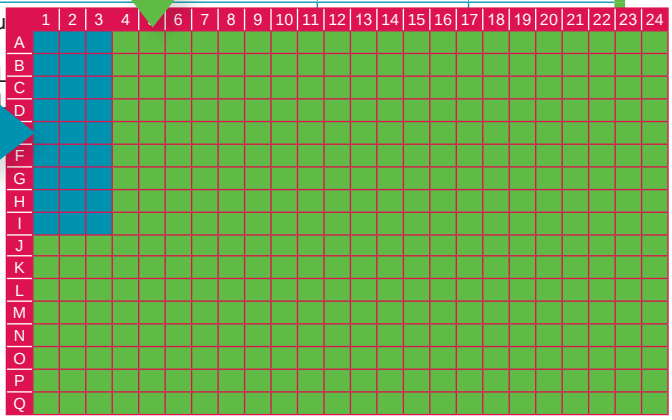
TO PREPARE SAMPLES:

- Each well requires 16 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (std 7) must be diluted diluent #5 (1X) or in your appropriate sample medium.

ASSAY MANUAL

	Standard (Std 0 - std 7)	Samples
Step 1 	Dispense 16 µL of each Human CTGF standard (Std 0 - std 7) into each standard well	Dispense 16 µL of each sample into each sample well
Step 2 	Add 2 µL of Human CTGF d2 antibody working solution to all wells	
Step 3 	Add 2 µL of Human CTGF Eu Cryptate antibody working solution to all wells	
Step 4 	Seal the plate and incubate overnight	
Step 5	Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	16 µL Std 0 (Negative control) 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well A1	Repeat Well A1	16 µL Sample 1 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well A4	Repeat Well A4
B	16 µL Std 1 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well B1	Repeat Well B1	16 µL Sample 2 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well B4	Repeat Well B4
C	16 µL Std 2 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well C1	Repeat Well C1	16 µL Sample 3 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well C4	Repeat Well C4
D	16 µL Std ... 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well D1	Repeat Well D1	16 µL Sample ... 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well D4	Repeat Well D4
E	16 µLStd ... 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well E1	Repeat Well E1	16 µL Sample ... 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well E4	Repeat Well E4
F	16 µL Std ... 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well F1	Repeat Well F1	16 µL Sample ... 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well F4	Repeat Well F4
G	16 µL Std ... 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well G1	Repeat Well G1	16 µL Sample ... 2 µL Human CT 2 µL Human CTG yptate	Repeat Well G4	Repeat Well G4
H	16 µL Std ... 2 µL Human CTGF-d2 2 µL Human CTGF-Eu Cryptate	Repeat Well H1	Repeat Well H1	16 µ 2 µL		



DATA REDUCTION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

3. Calculate the delta ratio of the acceptor and donor emission signals for each individual well. The Standard 0 (Negative control) plays the role of an internal assay control.

$$\text{delta Ratio} = \text{Ratio Standard or sample} - \text{Ratio Standard 0}$$

For more information about data reduction, please visit www.revvity.com

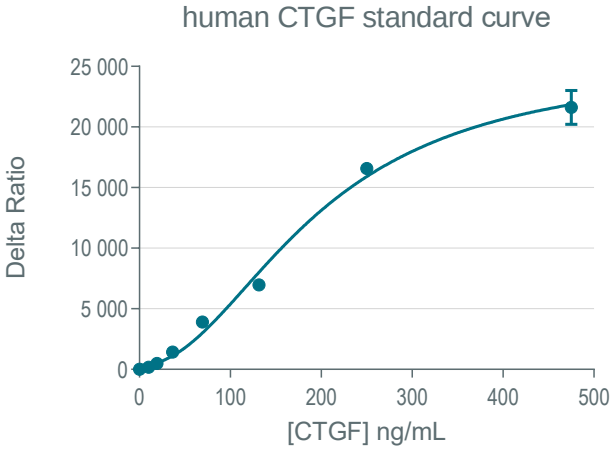
RESULTS

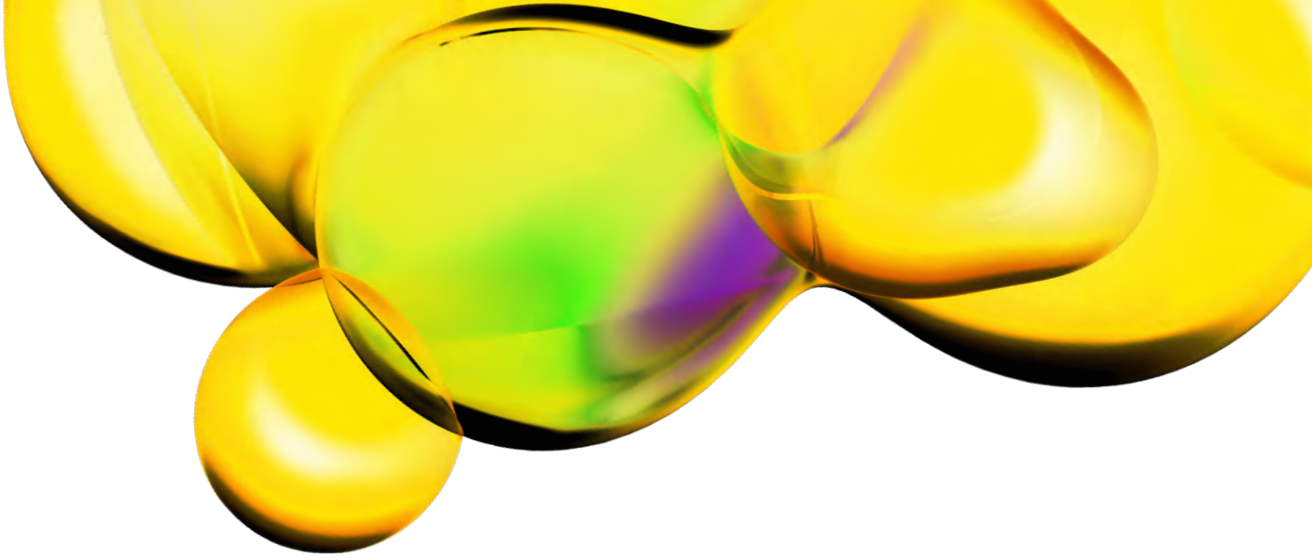
This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

Standard curve fitting with the 4 Parameter Logistic (4PL) model (with $1/Y^2$ weighting):

	Ratio (1)	CV (2)	Delta Ratio
Standard 0 - Negative control	576	0.4%	0
Standard 1 - 10.1 ng/mL	746	4.3%	170.5
Standard 2 - 19.2 ng/mL	1074	8.4%	498.5
Standard 3 - 36.45 ng/mL	1997	3.7%	1422
Standard 4 - 69.3 ng/mL	4482	8.4%	3907
Standard 5 - 131.6 ng/mL	7540	5.7%	6964
Standard 6 - 250 ng/mL	17149	1.5%	16573
Standard 7 - 475 ng/mL	22195	6.5%	21620





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