



CTGF KITS

PROTOCOL

Part # 64CTGFPEG & 64CTGFPEH

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

Revision: 01-July 2020

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.

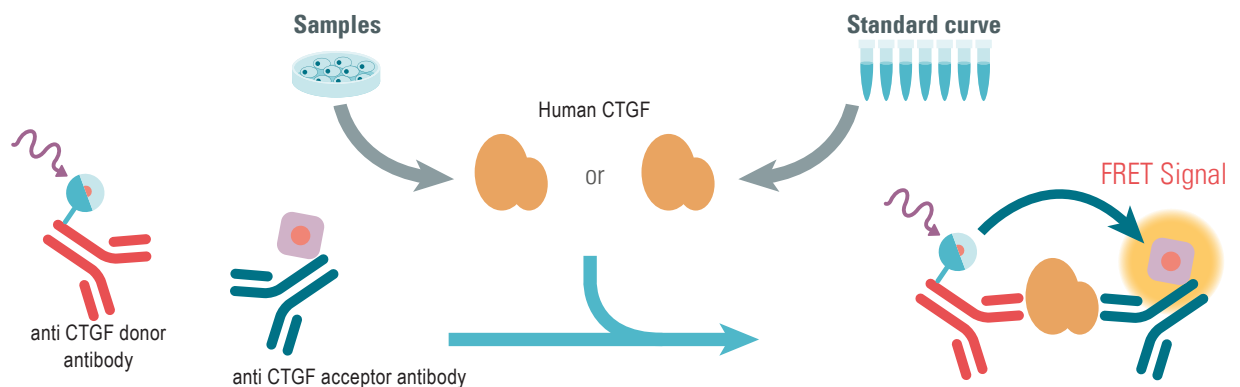
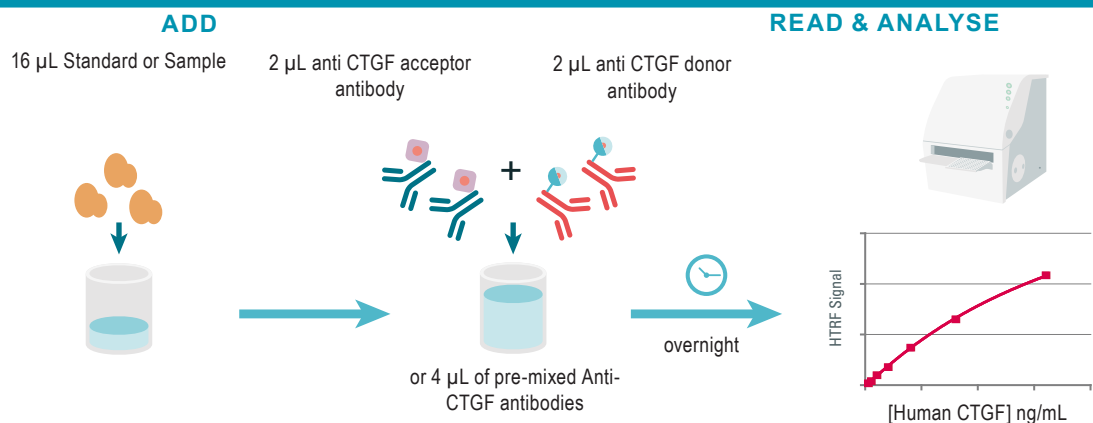


Figure 1: Principle of HTRF Human CTGF sandwich assay.

PROTOCOL 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.cisbio.com/compatible-readers

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

For more information about microplate recommendations, please visit our website at: cisbio.com/microplates-recommendations

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.



Reagents

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 .






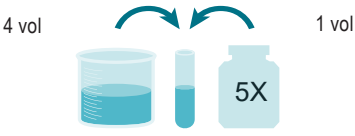
Volume of CTGF standard aliquots should not be under 10 µL.

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.

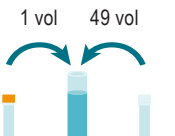
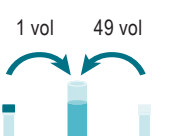

TO PREPARE REAGENT STOCK SOLUTIONS:

| 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.

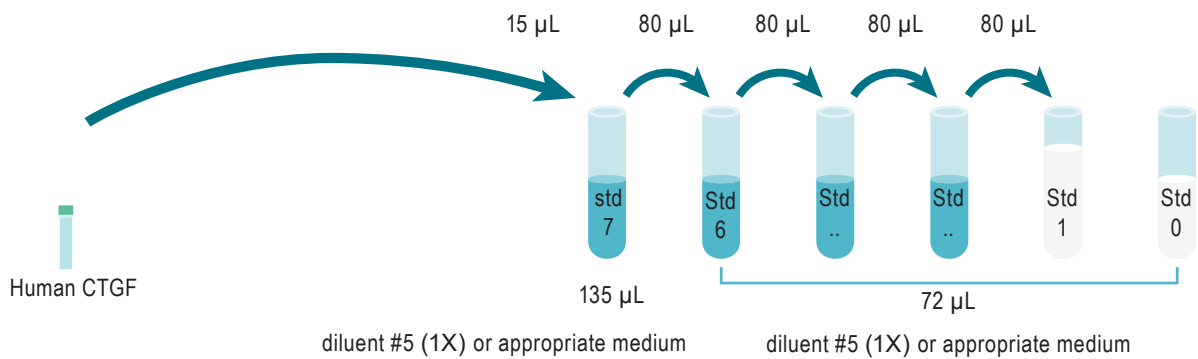
A recommended standard dilution procedure is listed and illustrated below:

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 PROTOCOL

| | | 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 | |

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 <http://www.cisbio.com/htrf-ratio-and-data-reduction>

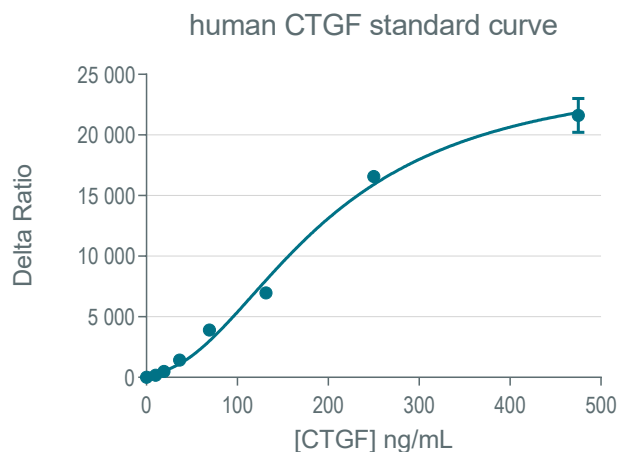
RESULTS

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/Y² 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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