



# HUMAN PRO-COLLAGEN TYPE I KITS

## PROTOCOL

### Part # 63ADK014PEG & 63ADK014PEH

**Test size#:** 500 tests (63ADK014PEG) and 10,000 tests (63ADK014PEH) - assay volume: 20  $\mu$ L

**Revision:** 05-May 2020

**Store at:** -60°C or below (63ADK014PEG); -60°C or below (63ADK014PEH)

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

## ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of human Pro-collagen Type I in cell/tissue culture supernatants and whole cells 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 Pro-Collagen is detected in a sandwich assay by using anti Human Pro-Collagen antibody labeled with Europium cryptate (donor), and anti Human Pro-Collagen 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 Pro-Collagen concentration.

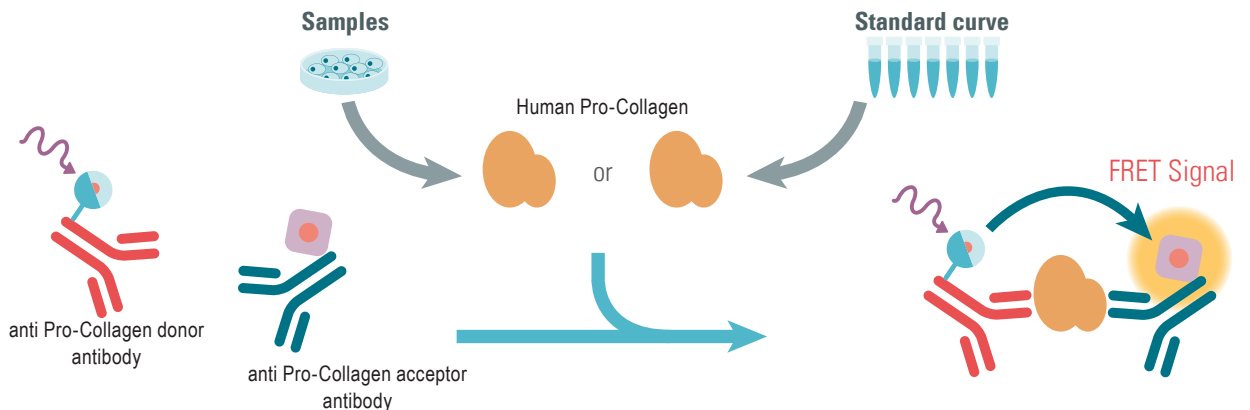
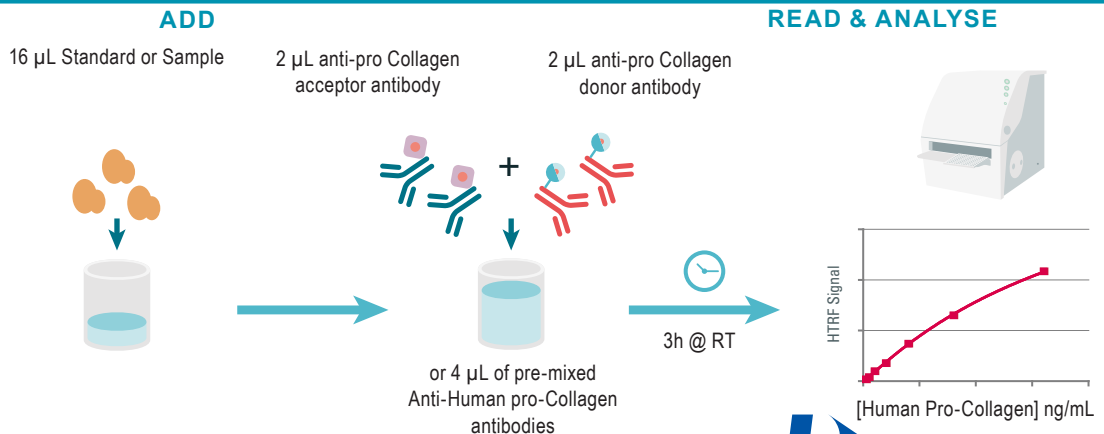


Figure 1: Principle of HTRF Human Pro-Collagen sandwich assay.

## PROTOCOL AT A GLANCE



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

**MATERIALS PROVIDED:**

| <b>KIT COMPONENTS</b>                   | <b>500 TESTS *<br/>CAT # 63ADK014PEG</b> | <b>10,000 TESTS *<br/>CAT # 63ADK014PEH</b> |
|---|--|---|
| Human Pro-Collagen Standard<br>Frozen   | 1 vial - 50 µL<br>10 µg/mL               | 1 vial - 50 µL<br>10 µg/mL                  |
| Human Pro-Collagen Eu Cryptate Antibody | 1 vial - 20 µL<br>Frozen - 50X           | 1 vial - 0.4 mL<br>Frozen - 50X             |
| Human Pro-Collagen d2 Antibody          | 1 vial - 20 µL<br>Frozen - 50X           | 1 vial - 0.4 mL<br>Frozen - 50X             |
| Diluent #5 **<br>5X                     | 1 vial<br>2 mL                           | 1 vial<br>10 mL                             |
| Detection buffer ***<br>ready-to-use    | 2 vials<br>1.5 mL<br>Detection Buffer #3 | 1 vial<br>50 mL<br>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](http://www.cisbio.com/compatible-readers)

- Small volume (SV) detection microplates - .

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






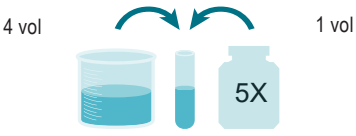
If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below .

**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 Pro-Collagen 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.**

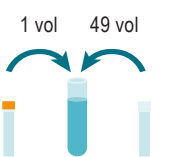
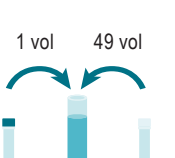

## TO PREPARE REAGENT STOCK SOLUTIONS:

| 500 TESTS KIT - 63ADK014PEG   |   |   | 10,000 TESTS KIT - 63ADK014PEH  |
|---|---|---|---|
| Anti-Human Pro-Collagen Eu Cryptate antibody  |   |   |   |
| Thaw the Human Pro-Collagen Eu Cryptate antibody . Mix gently.<br>This 50X stock solution can be frozen and stored at -60°C or below.   |  |  | Thaw the Human Pro-Collagen Eu Cryptate antibody . Mix gently.<br>This 50X stock solution can be frozen and stored at -60°C or below.   |
| Anti-Human Pro-Collagen d2 antibody   |   |   |   |
| Thaw the Human Pro-Collagen d2 antibody . Mix gently.<br>This 50X stock solution can be frozen and stored at -60°C or below.  |  |  | Thaw the Human Pro-Collagen d2 antibody . Mix gently.<br>This 50X stock solution can be frozen and stored at -60°C or below.  |
| Human Pro-Collagen Standard   |   |   |   |
| Thaw the Human pro-Collagen standard solution in order to obtain a 10 µg/mL stock solution. Mix gently.   |  |   | Thaw the Human pro-Collagen standard solution in order to obtain a 10 µg/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 Pro-Collagen-Eu Cryptate Antibody and 2 µL of Human Pro-Collagen-d2 Antibody.

Prepare the two antibody solutions in separate vials.

| 500 TESTS KIT - 63ADK014PEG  |   |  | 10,000 TESTS KIT - 63ADK014PEH   |
|--|---|--|--|
| Human Pro-Collagen Eu Cryptate antibody  |   |  |  |
| Dilute 50-fold the 50X stock solution (thawed reagent) of human Pro-Collagen 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 human human Pro-Collagen 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 Pro-Collagen d2 antibody   |   |  |  |
| Dilute 50-fold the 50X stock solution (thawed reagent) of human Human Pro-Collagen-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 human Human Pro-Collagen 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  $\mu\text{L}$  of standard.
- Dilute the standard stock solution serially with diluent #5 (1X) or if the sample to test is a cell supernatant, replace the diluent by culture medium
- (serum free and supplemented with BSA).
- 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.

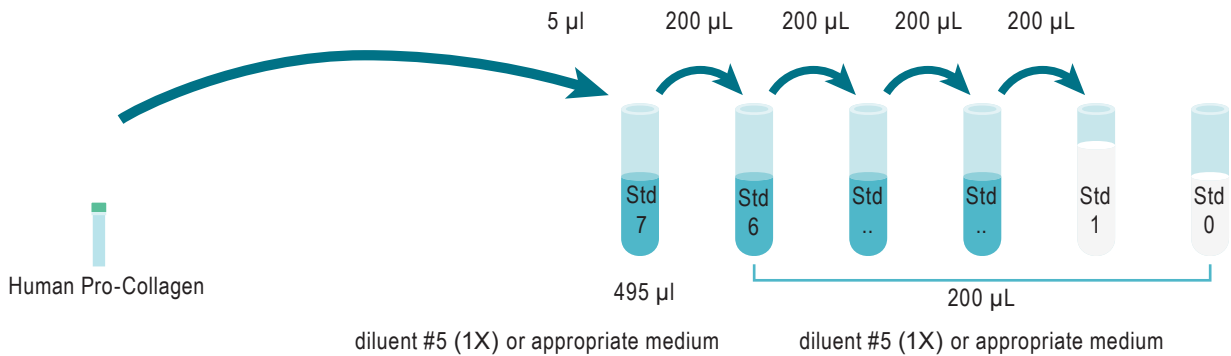
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 100-fold with diluent #5 (1X) to prepare high standard (Std 7): e.g. take 5  $\mu\text{L}$  of standard stock solution and add it to 495  $\mu\text{L}$  of diluent #5 (1X). Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:

- Dispense 200  $\mu\text{L}$  of diluent #5 (1X) in each vial from Std 6 to Std 0.
- Add 200  $\mu\text{L}$  of standard to 200  $\mu\text{L}$  of diluent #5 (1X), mix gently and repeat the 1/2 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   | HUMAN PRO-COLLAGEN WORKING SOLUTIONS (ng/mL) |
|-------------------------|--|--|
| Standard Stock solution | Thawed stock solution  | 10,000                                       |
| Standard 7              | 5 $\mu\text{L}$ standard stock solution + 495 $\mu\text{L}$ Diluent 1X | 100  |
| Standard 6              | 200 $\mu\text{L}$ standard 7 + 200 $\mu\text{L}$ Diluent 1X            | 50   |
| Standard 5              | 200 $\mu\text{L}$ standard 6 + 200 $\mu\text{L}$ Diluent 1X            | 25   |
| Standard 4              | 200 $\mu\text{L}$ standard 5 + 200 $\mu\text{L}$ Diluent 1X            | 12.5   |
| Standard 3              | 200 $\mu\text{L}$ standard 4 + 200 $\mu\text{L}$ Diluent 1X            | 6.25   |
| Standard 2              | 200 $\mu\text{L}$ standard 3 + 200 $\mu\text{L}$ Diluent 1X            | 3.1  |
| Standard 1              | 200 $\mu\text{L}$ standard 2 + 200 $\mu\text{L}$ Diluent 1X            | 1.56   |
| Standard 0              | 200 $\mu\text{L}$ Diluent 1X   | 0  |

## TO PREPARE SAMPLES:

- Each well requires 16  $\mu\text{L}$  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)

## ASSAY PROTOCOL

|   | Standard (Std 0 - Std 7)   | Samples  |
|---|--|--|
| <b>Step 1</b><br>  | Dispense 16 $\mu\text{L}$ of each Human Pro-Collagen standard (Std 0 - Std 7) into each standard well  | Dispense 16 $\mu\text{L}$ of each sample into each sample well |
| <b>Step 2</b><br>  | Add 2 $\mu\text{L}$ of Human Pro-Collagen d2 antibody working solution to all wells  |  |
| <b>Step 3</b><br>  | Add 2 $\mu\text{L}$ of Human Pro-Collagen Eu Cryptate antibody working solution to all wells   |  |
| <b>Step 4</b><br>  | Seal the plate and incubate 3h @ RT<br>Incubation time beyond 3h will result in a loss of dynamic range at the highest concentration of standard |  |
| <b>Step 5</b><br> | 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 F which reflects the signal to background of the assay. The negative control (Standard 0) plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample} - \text{Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

For more information about data reduction, please visit <http://www.cisbio.com/htrf-ratio-and-data-reduction>

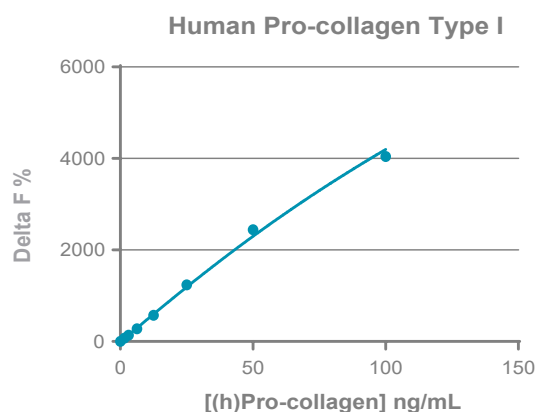
## RESULTS

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:

|                               | Ratio <sup>(1)</sup> | CV <sup>(2)</sup> | Delta F% <sup>(3)</sup> |
|-------------------------------|----------------------|-------------------|-------------------------|
| Standard 0 - Negative control | 784                  | 0%                | 0%                      |
| Standard 1 - 1.56 ng/mL       | 1,364                | 5%                | 74%                     |
| Standard 2 - 3.1 ng/mL        | 1,854                | 3%                | 136%                    |
| Standard 3 - 6.25 ng/mL       | 2,956                | 3%                | 277%                    |
| Standard 4 - 12.5 ng/mL       | 5,280                | 2%                | 573%                    |
| Standard 5 - 25 ng/mL         | 10,470               | 2%                | 1,235%                  |
| Standard 6 - 50 ng/mL         | 19,930               | 3%                | 2,442%                  |
| Standard 7 - 100 ng/mL        | 32,488               | 0%                | 4,044%                  |



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