

HUMAN CCL2 KITS

Part # 62HCCL2PEG & 62HCCL2PEH

Test size: 500 tests (62HCCL2PEG), 10,000 tests (62HCCL2PEH) - assay volume: 20 μ L

Revision: #08 of September 2023

Store at: $\leq -16^{\circ}\text{C}$

This product is intended for research purposes only. The product is not intended to be used for therapeutic or diagnostic purposes.

ASSAY PRINCIPLE

Revvity human CCL2 assay is only intended for the quantitative measurement of CCL2 in supernatant using HTRF[®] technology. The assay is compatible with human samples, and is highly specific for CCL2.

CCL2 is detected in a sandwich assay format using 2 different specific antibodies, one labeled with Europium Cryptate (donor) and the second with d2 (acceptor).

The detection principle is based on HTRF[®] technology. When the labelled antibodies bind to the same antigen, 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). The two antibodies bind to the CCL2 present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the CCL2 concentration. (Fig. 1).

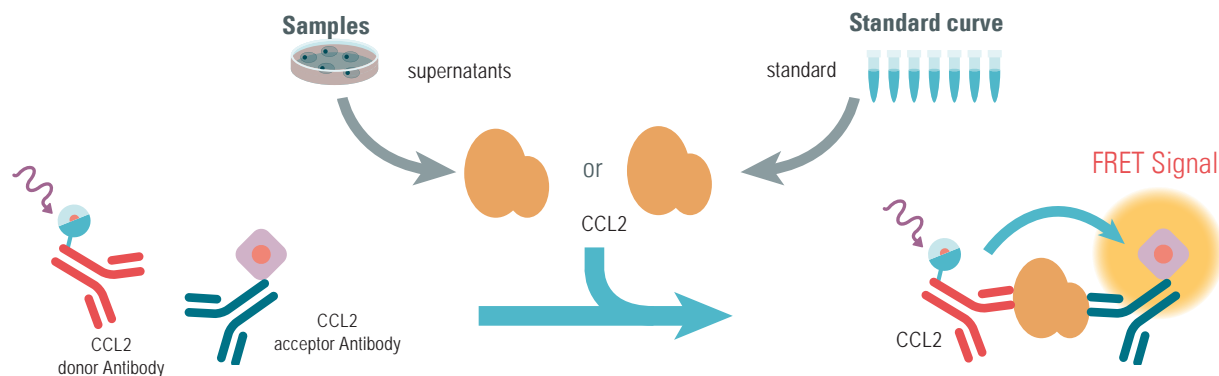
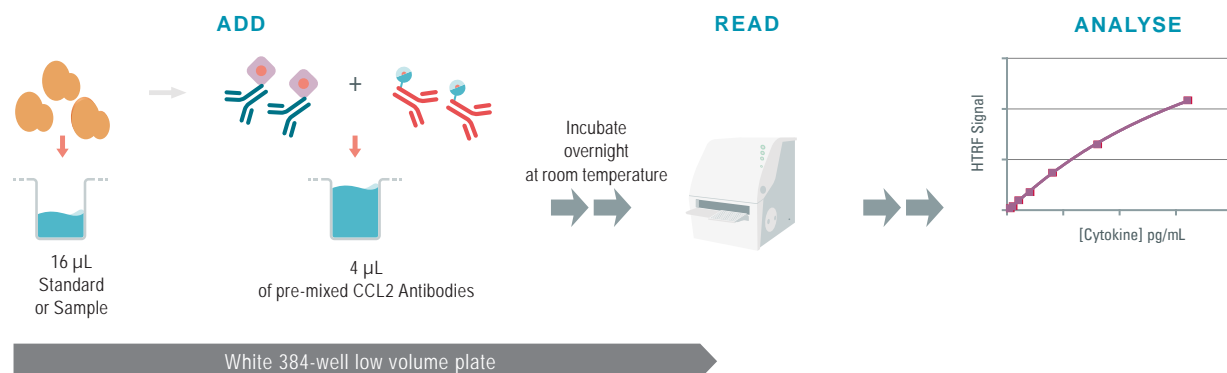


Figure 1: Principle of the HTRF CCL2 sandwich assay.

MANUAL AT A GLANCE



Make sure to use the set-up for Eu^{3+} Cryptate.

For more information about set-up and compatible HTRF[®] readers, please visit our website at: www.revvity.com

MATERIALS:

| KIT COMPONENTS | 500 TESTS CAT # 62HCCL2PEG | 10,000 TESTS CAT # 62HCCL2PEH |
|--|-------------------------------|----------------------------------|
| CCL2 Standard Lyophilized | 2 vials | 2 vials |
| CCL2 Eu Cryptate Antibody Frozen - 20 X | 1 vial - 50 μ L | 1 vial - 1 mL |
| CCL2 d2 Antibody Frozen - 20 X | 1 vial - 50 μ L | 1 vial - 1 mL |
| Diluent* #5 5X | 1 vial 2 mL | 1 vial 10 mL |
| Detection Buffer** #3 ready-to-use | 2 vials 1.5 mL | 1 vial 50 mL |

* To prepare working standard solutions, culture medium can be an alternative the diluent.

** The Detection Buffer is used to prepare working solutions of acceptor and donor reagents.

FOR READING, AN HTRF®-CERTIFIED READER IS NEEDED.

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

PURCHASE SEPARATELY

96-well or 384-well small volume (SV) detection microplates - For more information about microplate recommendations, please visit our website at: www.revvy.com

STORAGE AND STABILITY

Store the kit at $\leq -16^{\circ}\text{C}$. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.

Once reconstituted, standard stock solution may be frozen, and can be thawed only once..

Once thawed, antibody solutions can be frozen once.

To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at $\leq -60^{\circ}\text{C}$.

Volume of standard and antibody aliquots should not be under 10 μ L.




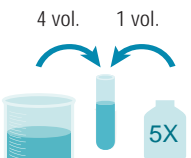
Thawed diluent and detection buffer can be stored at 2-8 $^{\circ}\text{C}$ on your premises.

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.
- Before use, allow all kit's reagents to warm up at room temperature then
 - homogenize buffer and diluent with a vortex
 - centrifuge (NEVER vortex) the antibodies to gather all liquid at the bottom of the vial
- It is recommended to filter buffers before use.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.

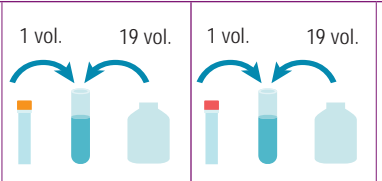
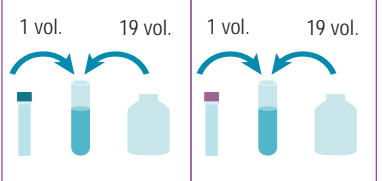
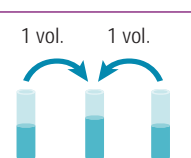
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE DILUENT, STANDARD & ANTIBODY STOCK SOLUTIONS:

| 500 TESTS | | 10,000 TESTS |
|--|---|--|
| CCL2 Eu Cryptate antibody | | |
| Thaw the CCL2 Eu Cryptate antibody. Centrifuge. This 20 X stock solution can be frozen and stored at $\leq -60^{\circ}\text{C}$. |  | Thaw the CCL2 Eu Cryptate antibody. Centrifuge. This 20 X stock solution can be frozen and stored at $\leq -60^{\circ}\text{C}$. |
| CCL2 d2 antibody | | |
| Thaw the CCL2 d2 antibody. Centrifuge. This 20 X stock solution can be frozen and stored at $\leq -60^{\circ}\text{C}$. |  | Thaw the CCL2 d2 antibody. Centrifuge. This 20 X stock solution can be frozen and stored at $\leq -60^{\circ}\text{C}$. |
| CCL2 Standard | | |
| Reconstitute the CCL2 standard with distilled water. Volume of reconstitution is indicated on the vial label. The reconstituted standard solution can be frozen and stored at -60°C or below. |  | Reconstitute the CCL2 standard with distilled water. Volume of reconstitution is indicated on the vial label. The reconstituted standard solution can be frozen and stored at -60°C or below |
| 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. |  | 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. |

TO PREPARE WORKING ANTIBODY SOLUTIONS:

Each well requires 4 μL of pre-mixed CCL2 antibodies. Prepare the two antibody solutions in separate vials.

| 500 TESTS | | 10,000 TESTS |
|--|---|---|
| CCL2 Eu Cryptate antibody | | |
| Dilute 20-fold the 20 X stock solution (thawed reagent) of CCL2 Eu Cryptate antibody with detection buffer #3: e.g. 10 μL of thawed Eu Cryptate antibody stock solution + 190 μL of detection buffer. |  | Dilute 20-fold the 20 X stock solution (thawed reagent) of CCL2 Eu Cryptate antibody with detection buffer #3: e.g. 10 μL of thawed Eu Cryptate antibody stock solution + 190 μL of detection buffer). |
| CCL2 d2 antibody | | |
| Dilute 20-fold the 20 X stock solution (thawed reagent) of CCL2 d2 antibody with detection buffer #3: e.g. 10 μL of thawed d2 antibody stock solution + 190 μL of detection buffer. |  | Dilute 20-fold the 20 X stock solution (thawed reagent) of CCL2 d2 antibody with detection buffer #3: e.g. 10 μL of thawed d2 antibody stock solution + 190 μL of detection buffer. |
| Antibody mix | | |
| Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents: e.g. 200 μL of d2 antibody + 200 μL of Eu Cryptate antibody |  | Pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents: e.g. 200 μL of d2 antibody + 200 μL of Eu Cryptate-antibody |

TO PREPARE WORKING STANDARD SOLUTIONS:

- Each well requires 16 μL of standard.
- Serially dilute the standard stock solution with diluent #5 or with the cell culture medium used to prepare your samples, supplemented with BSA or 10% FCS.
- **Due to the stability of the CCL2, it is mandatory to prepare the standard curve just before the assay.**
- 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.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

1. Reconstitute the standard vial with the volume indicated on the vial label using distilled water.

2. Prepare the following dilutions:

- Dilute the reconstituted standard stock solution 3-fold with diluent or with cell culture medium.

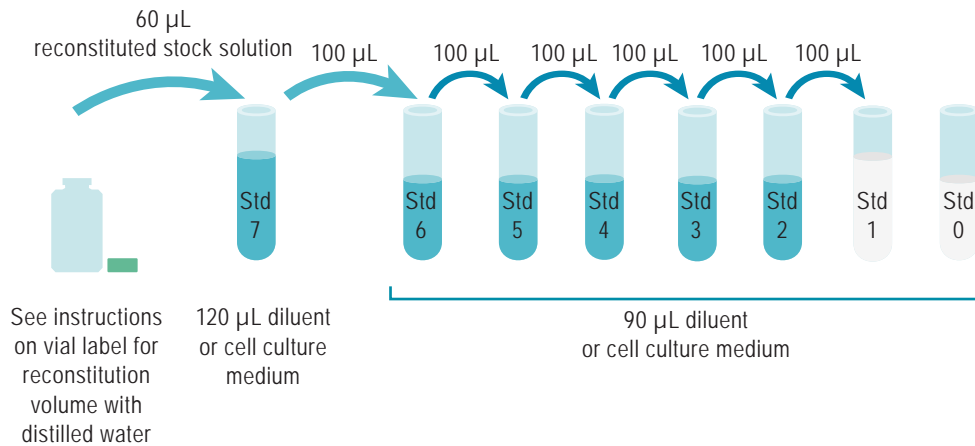
In practice: take 60 μL of stock solution and add it to 120 μL of diluent or cell culture medium. Mix gently. This yields the high standard (Std 7: 3000 pg/mL) for the top of the curve.

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

- Dispense 90 μL of diluent or cell culture medium into each vial from Std 6 to Std 0

- Add 100 μL of standard to 90 μL of diluent or cell culture medium, mix gently and repeat the 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 or appropriate culture medium alone.



| STANDARD | SERIAL DILUTIONS | WORKING SOLUTIONS |
|-------------------------|--|-------------------|
| Standard Stock solution | Reconstitute the vial following the indications given on the vial label | 9 ng/mL |
| Standard 7 | 60 μL reconstituted standard stock solution + 120 μL diluent | 3000 pg/mL |
| Standard 6 | 100 μL Standard 7 + 90 μL diluent | 1579 pg/mL |
| Standard 5 | 100 μL Standard 6 + 90 μL diluent | 831 pg/mL |
| Standard 4 | 100 μL Standard 5 + 90 μL diluent | 437 pg/mL |
| Standard 3 | 100 μL Standard 4 + 90 μL diluent | 230 pg/mL |
| Standard 2 | 100 μL Standard 3 + 90 μL diluent | 121 pg/mL |
| Standard 1 | 100 μL Standard 2 + 90 μL diluent | 64 pg/mL |
| Standard 0 | 90 μL diluent | 0 |

TO PREPARE SAMPLES:

- Each well requires 16 μL of sample.
- Just after their collection, store the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at $\leq -60^\circ\text{C}$. Avoid multiple freeze/thaw cycles.
- All samples with a concentration above the highest standard (Std 7) must be diluted in diluent #5 or in your cell culture medium.

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 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.

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

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

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

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

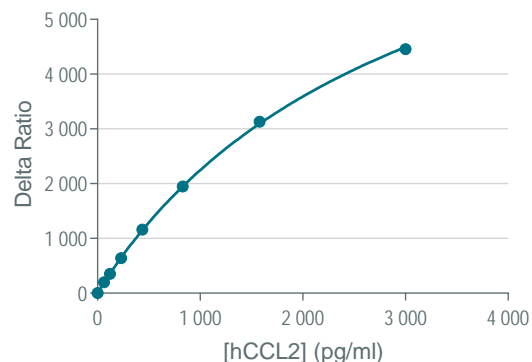
RESULTS

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 1/y²)* model

* For more information about curve fitting please visit www.revvy.com

| | | Ratio (1) | delta R (2) | CV% (3) |
|------------|------------------|-----------|-------------|---------|
| Standard 0 | Negative control | 633 | 0 | 3% |
| Standard 1 | 64 pg/mL | 829 | 196 | 2% |
| Standard 2 | 121 pg/mL | 985 | 352 | 2% |
| Standard 3 | 230 pg/mL | 1273 | 640 | 1% |
| Standard 4 | 437 pg/mL | 1795 | 1162 | 0% |
| Standard 5 | 831 pg/mL | 2583 | 1950 | 1% |
| Standard 6 | 1579 pg/mL | 3766 | 3133 | 2% |
| Standard 7 | 3000 pg/mL | 5089 | 4456 | 1% |



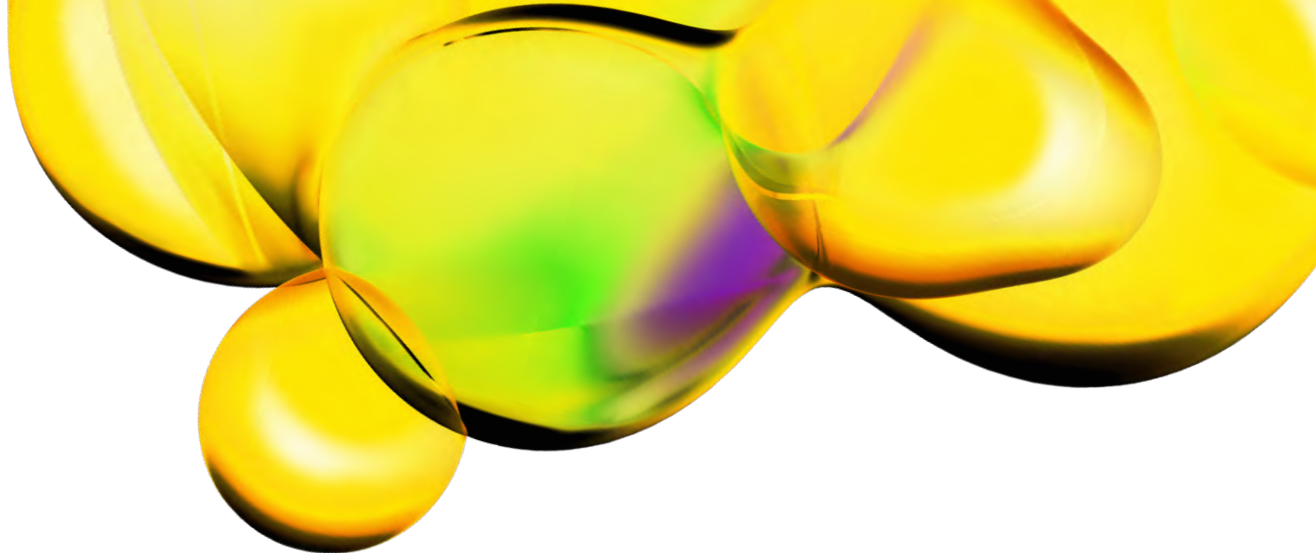
ANALYTICAL ASSAY PERFORMANCE

| | Diluent | DMEM | RPMI |
|---|-------------------------------|----------|----------|
| Assay range (pg/mL**) | 90 pg/mL to 3000 pg/mL | | |
| Limit of detection (LoD*) = Std 0 mean + 2 SD | 20 pg/mL | 28 pg/mL | 20 pg/mL |
| Limit of quantification (LoQ*) | 90 pg/mL | | |
| Incubation time | overnight at room temperature | | |

**NIBSC (92/794) value (IU/mL) = 0,9 x HTRF hCCL2 value (pg/mL)

* The analytical sensitivity was calculated from data obtained with an HTRF compatible reader after overnight incubation, this may vary from one HTRF compatible reader to another.

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