# HUMAN PERFORIN-1 KITS

PROTOCOL

**Part # 63ADK100PEG & 63ADK100PEH**

**Test size#:** 500 tests (63ADK100PEG) and 10,000 tests (63ADK100PEH) - assay volume: 20 µL

**Revision:** 04-May 2020

**Store at:** -60°C or below (63ADK100PEG); -60°C or below (63ADK100PEH)

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

## ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of human Perforin-1 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, Perforin-1 is detected in a sandwich assay by using anti Perforin-1 antibody labeled with Europium cryptate (donor), and anti Perforin-1 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 Perforin-1 concentration.

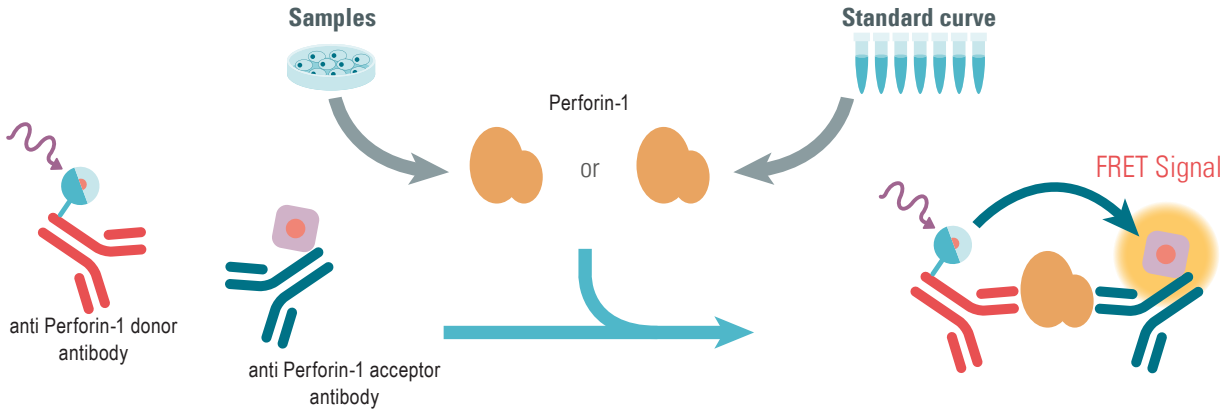
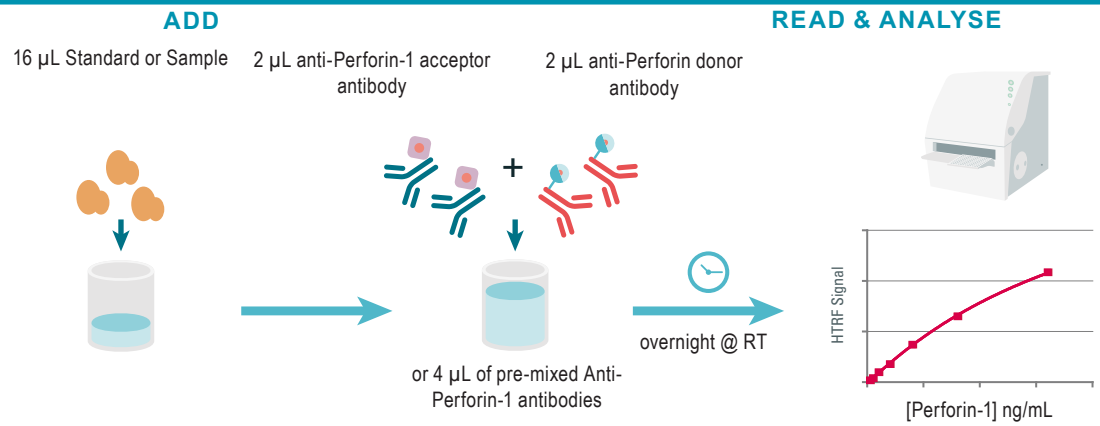



Figure 1: Principle of HTRF Perforin-1 sandwich assay.

## PROTOCOL AT A GLANCE




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

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## MATERIALS PROVIDED:

| KIT COMPONENTS | 500 TESTS * CAT # 63ADK100PEG | 10,000 TESTS * CAT # 63ADK100PEH |
|---|---|---|
| Perforin-1 Standard Frozen | 1 vial - 10 µL 10 µg/mL | 1 vial - 10 µL 10 µg/mL |
| Perforin-1 Eu Cryptate Antibody | 1 vial - 20 µL Frozen - 50X | 1 vial - 0.4 mL Frozen - 50X |
| Perforin-1 d2 Antibody | 1 vial - 20 µL Frozen - 50X | 1 vial - 0.4 mL Frozen - 50X |
| Diluent ** ready-to-use | 1 vial 20 mL | 1 vial 20 mL |
| Detection buffer *** ready-to-use | 1 vial 2 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 - .

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

## STORAGE AND STABILITY

Reagents

Store the kit at -60°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label. Detection buffer is 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 .

## 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.
- Perforin-1 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.

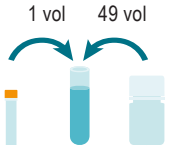
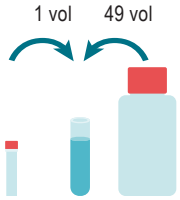
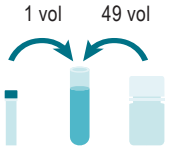
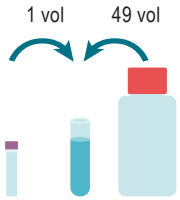
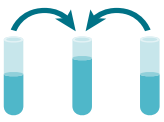
## TO PREPARE REAGENT STOCK SOLUTIONS:

| 500 TESTS KIT - 63ADK100PEG | | 10,000 TESTS KIT - 63ADK100PEH |
|---|---|---|
| Anti-Perforin-1 Eu Cryptate antibody | | |
| Thaw the Perforin-1 Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. |  | Thaw the Perforin-1 Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. |
| Anti-Perforin-1 d2 antibody | | |
| Thaw the Perforin-1 d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. |  | Thaw the Perforin-1 d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. |
| Perforin-1 Standard | | |
| Thaw the Perforin-1 Standard in order to obtain a 10 µg/mL stock solution. Mix gently. This stock solution can be aliquoted, frozen and stored at -60°C or below. |  | Thaw the Perforin-1 Standard in order to obtain a 10 µg/mL stock solution. Mix gently. This stock solution can be aliquoted, frozen and stored at -60°C or below. |
| Diluent | | |
| The diluent is ready-to-use | | The diluent is ready-to-use |
| 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 Perforin-1-Eu Cryptate Antibody and 2 µL of Perforin-1-d2 Antibody.

Prepare the two antibody solutions in separate vials.

| 500 TESTS KIT - 63ADK100PEG | | 10,000 TESTS KIT - 63ADK100PEH |
|---|---|---|
| Perforin-1 Eu Cryptate antibody | | |
| Dilute 50-fold the 50X stock solution (thawed reagent) of human-Perforin-1 Eu Cryptate antibody with the detection buffer: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer (e.g., 20 µL of thawed Eu Cryptate antibody stock solution + 980 µL of Detection Buffer). |  |  Dilute 50-fold the 50X stock solution (thawed reagent) of human Perforin-1 Eu Cryptate antibody with the detection buffer: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer (eg., 40µl of thawed Eu Cryptate antibody stock solution + 1960µl of detection buffer). |
| Perforin-1 d2 antibody | | |
| Dilute 50-fold the 50X stock solution (thawed reagent) of Human Perforin-1 d2 antibody with the detection buffer: add 1 volume of d2 antibody stock solution in 49 volumes of detection buffer(e.g., 20 µL of thawed d2 antibody stock solution + 980 µL of Detection Buffer). |  |  Dilute 50-fold the 50X stock solution (thawed reagent) of Human Perforin-1 d2 antibody with the detection buffer: add 1 volume of d2 antibody stock solution in 49 volumes of detection buffer (eg., 40µl of thawed d2 antibody stock solution + 1960µl of detection buffer). |
| 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
- 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:

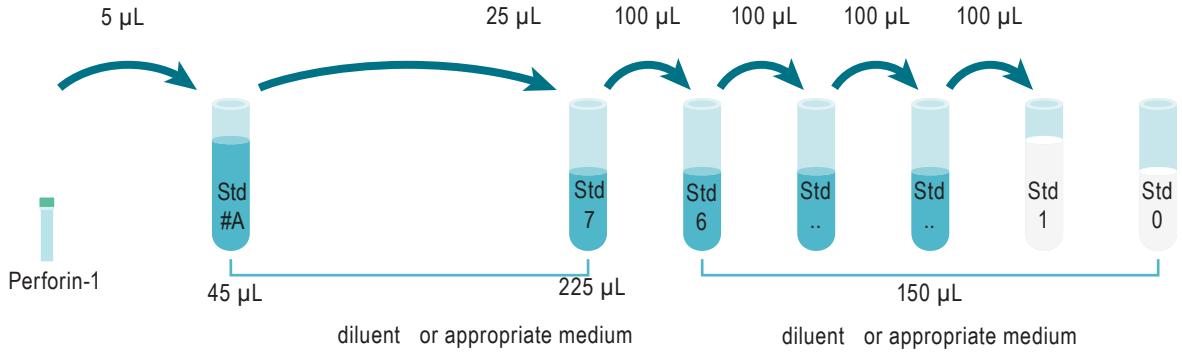
Dilute the standard stock solution 10-fold with diluent; this yields the Intermediate Standard solution #A (1, 000 ng/mL). e.g: take 5 µL of standard stock solution and add it to 45 µL of diluent. Mix gently.

Dilute the Intermediate Standard dilution #A 10-fold with diluent to prepare high standard (Std 7): e.g. take 25 µL of Intermediate Standard dilution #A and add it to 225 µL of diluent . Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/2.5 serial dilutions as follows:

- Dispense 150 µL of diluent in each vial from Std 6 to Std 0.
- Add 100 µL of standard to 150 µL of diluent , mix gently and repeat the 1/2.5 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 | HUMAN PERFORIN-1 WORKING SOLUTIONS (ng/ml) |
|---|---|---|
| Standard Stock solution | Thawed stock solution | 10,000 |
| Intermediate standard solution #A | 5 µL Standard stock solution + 45 µL Diluent | 1,000 |
| Standard 7 | 25µL Intermediate Standard Solution #A + 225 µL Diluent | 100 |
| Standard 6 | 100 µL standard 7 + 150 µL Diluent | 40 |
| Standard 5 | 100 µL standard 6 + 150 µL Diluent | 16 |
| Standard 4 | 100 µL standard 5 + 150 µL Diluent | 6.4 |
| Standard 3 | 100 µL standard 4 + 150 µL Diluent | 2.56 |
| Standard 2 | 100 µL standard 3 + 150 µL Diluent | 1.02 |
| Standard 1 | 100 µL standard 2 + 150 µL Diluent | 0.41 |
| Standard 0 | 100 µL Diluent | 0 |

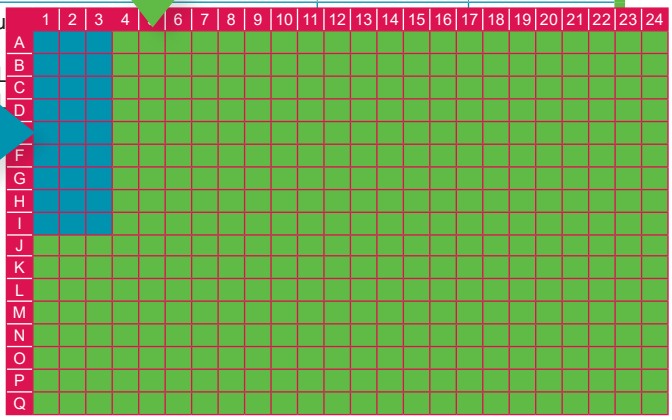
## TO PREPARE SAMPLES:

- 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

## ASSAY PROTOCOL

| | Standard (Std 0 - Std 7) | Samples |
|---|---|---|
| Step 1  | Dispense 16 µL of each Perforin-1 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 Perforin-1 d2 antibody working solution to all wells | |
| Step 3  | Add 2 µL of Perforin-1 Eu Cryptate antibody working solution to all wells | |
| Step 4  | Seal the plate and incubate overnight @ RT | |
| 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)<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well A1 | Repeat Well A1 | 16 µL Sample 1<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well A4 | Repeat Well A4 |
| B | 16 µL Std 1<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well B1 | Repeat Well B1 | 16 µL Sample 2<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well B4 | Repeat Well B4 |
| C | 16 µL Std 2<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well C1 | Repeat Well C1 | 16 µL Sample 3<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well C4 | Repeat Well C4 |
| D | 16 µL Std ...<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well D1 | Repeat Well D1 | 16 µL Sample ...<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well D4 | Repeat Well D4 |
| E | 16 µLStd ...<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well E1 | Repeat Well E1 | 16 µL Sample ...<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well E4 | Repeat Well E4 |
| F | 16 µL Std ...<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well F1 | Repeat Well F1 | 16 µL Sample ...<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well F4 | Repeat Well F4 |
| G | 16 µL Std ...<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well G1 | Repeat Well G1 | 16 µL Sample ...<br><br>2 µL Perforin-1<br>2 µL Perforin-1-Eu te | Repeat Well G4 | Repeat Well G4 |
| H | 16 µL Std ...<br><br>2 µL Perforin-1-d2<br>2 µL Perforin-1-Eu Cryptate | Repeat Well H1 | Repeat Well H1 | 16 µ<br><br>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.

delta Ratio = Ratio Standard or sample - Ratio Standard 0

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

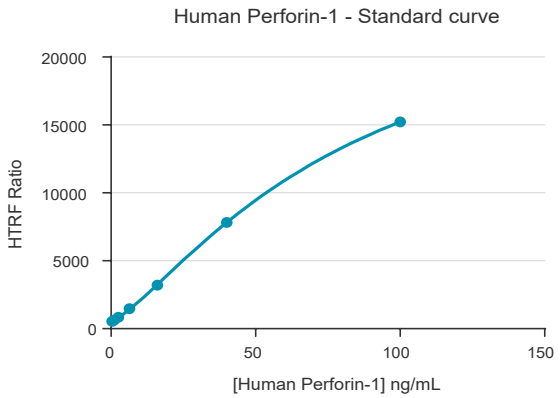
## RESULTS

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 1/Y²) model:

| | Ratio (1) | CV (2) |
|---|---|---|
| Standard 0 - Negative control | 489 | 0% |
| Standard 1 - 0.41 ng/mL | 535 | 1% |
| Standard 2 - 1.02 ng/mL | 629 | 4% |
| Standard 3 - 2.56 ng/mL | 847 | 6% |
| Standard 4 - 6.4 ng/mL | 1,476 | 3% |
| Standard 5 - 16 ng/mL | 3,205 | 3% |
| Standard 6 - 40 ng/mL | 7,832 | 4% |
| Standard 7 - 100 ng/mL | 15,226 | 0% |



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