

## Human Perforin ELISA Kit

**Catalog No:** CKH331

**Lot No.:** TBD

**Size:** 5 plates (5 x 96 tests)

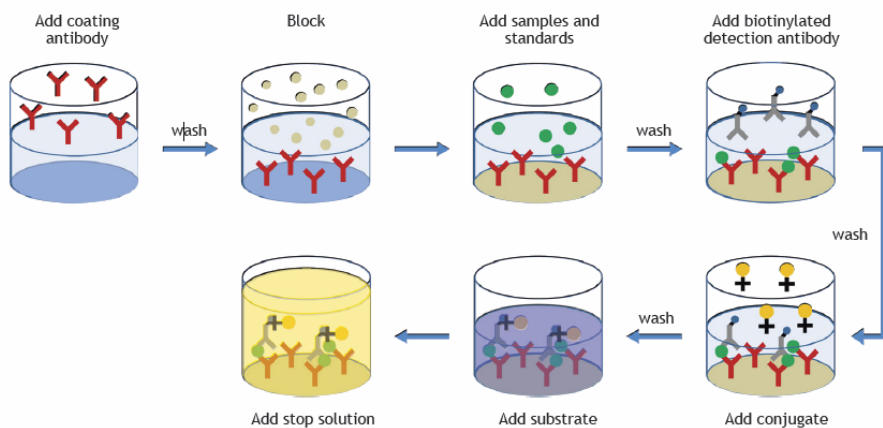
**Expiration Date:** TBD

NOTE: this is a sample protocol which is subject to variation by Lot Number. Refer to the protocol inserted in your package for the current lot number specifications and expiration date or contact our technical support at [tech@cellsciences.com](mailto:tech@cellsciences.com)

Specificity:	Native human perforin
Assay Sensitivity:	64 pg/mL
Assay Range:	64 – 4096 pg/mL
Sample Type:	Plasma, serum and culture supernatant

### Principle of the Test

The ELISA assay is a simple and sensitive sandwich immunoassay for the determination of cytokine, chemokine and granzyme levels in biological fluids such as cell culture supernatant, plasma or serum. The assay utilizes a coating antibody specific for the analyte of interest (e.g. cytokine, chemokine or granzyme) coated on the wells of a 96-well microtiter plate. The wells are washed and blocked. Standards and samples are added to the wells and any analyte present binds to the immobilized coating antibody. After washing off excess/unbound materials, the bound analyte is allowed to associate with a biotinylated detection antibody. The wells are washed again and a streptavidin-HRP (SPP) conjugate is added: the wells are washed again and a chromogenic substrate (TMB) is introduced, which produces a blue-colored product of which the intensity is related to the amount of analyte in the sample. The optical density (OD) can now be read at 370 or 655 nm (not recommended as the OD will change over time). A sulfuric acid solution is added to stop the enzymatic reaction (changing the color to yellow) and OD is read at 450 nm.



### Warnings and Precautions



## Human Perforin ELISA Kit

This kit is designed for research use only, and not for diagnostic or therapeutic procedures.

When blood components or other biological materials are used, then please note that all these materials should be considered as potentially infectious and handled with the usual precautions under Bio-Hazard conditions. Follow universal precautions as established by the US government agencies, Centers for Disease Control and Prevention and Occupational Safety and Health Administration, when handling and disposing of (potentially) infectious waste.

### Reagents and Materials Supplied with the Kit:

Items	Quantity (for 5 plates)	Storage conditions
CKH331-A Coating Antibody (lyophilized)	1 vial	4 °C
CKH331 –B Standard (lyophilized)	5 vials	4 °C
CKH331 –C Biotinylated Detection Antibody (lyophilized)	1 vial	4 °C
CKH331-D SPP Conjugate (lyophilized)	1 vial	-20 °C in the dark
CKH331-E BSA Stock Solution (10%)	2 vials (2 x 12 mL)	4°C
CKH331-F Cytokine Stabilization Buffer (CSB)	1 vial (5 mL)	4°C
CKH331-G Tween 20	1 vial (5 mL)	RT in the dark
CKH331-H TMB Substrate Solution	2 vials (2 x 30 mL)	4 °C in the dark
CKH331-I Stop Solution (0.175 M H <sub>2</sub> SO <sub>4</sub> )	2 vials (2 x 30 mL)	4 °C
CKH331-P Elisa Plates	8 plates	RT
CKH331-Z Adhesive cover slips	10 slips	RT

### Materials/Reagents Required but not Provided:

- PBS, pH 7.4
- Sterile distilled water.
- Pipetting devices for the accurate delivery of volume required for the assay performance.
- Tubes and containers/plates to make solutions.
- 37 °C incubator.
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
- Reading device for microtiter-plate set to 370, 450 and/or 655 nm.

### Specimen Collection and Handling:

Specimens should be clear, non-hemolyzed and non-lipemic. Excessive hemolysis and the presence of large clots or microbial growth in the sample may interfere with the performance of the test.

- **Cell culture supernatant:** remove any particulate matter by centrifugation.



## Human Perforin ELISA Kit

- **Serum:** use a clot tube and allow sample to clot for 30-45 minutes at RT, then centrifuge for 10-15 minutes at 1,000 to 2,000 x g (RT) and collect serum immediately.
- **Plasma:** collect plasma by using anticoagulant, such as EDTA or heparin. Mix well immediately after collection. Centrifuge for 10-15 minutes at 1,000-2,000 x g at RT and collect plasma.

Samples should be aliquoted and stored frozen at -20 °C to -80 °C to prevent cytokine degradation. If samples are run within 24 hours, they may be stored at 2-8 °C. Avoid repeated freeze-thaw cycles. Do not heat serum or plasma samples. Prior to assay, frozen samples should be completely thawed and mixed well.

**Note:** Specimen collection from humans and non-human primates should be carried out in accordance with the Clinical and Laboratory Standards Institute document M29-T2, "Protection of laboratory workers from infectious diseases transmitted by blood and tissue".

### Preparation, Storage and Stability:

#### PBS

- PB stock: dissolve 96.0 g of Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O plus 17.5 g of KH<sub>2</sub>PO<sub>4</sub> in 1 L distilled water, adjust pH to 7.4 and filter solution (0.2 µm). Store solution at RT. The PB stock is stable for at least 6 months when kept sterile.
- PBS: add 10 mL of the PB stock and 8.8 g of NaCl to 1 L distilled water. It is strongly recommended to prepare PBS freshly each day. Alternatively, when PBS is prepared in advance, the solution should be filter sterilized (0.2 µm) or autoclaved.

#### Wash buffer

- PBS containing 0.05% Tween-20 (add 500 µL of Tween-20 to 1 L PBS). The volume is dependent on the washing procedure (manual or automatic washing).

#### Blocking buffer

- PBS containing 1% BSA. For one ELISA plate, mix 2 mL BSA stock solution (10%) is gently but thoroughly mixed with 18 mL PBS.

#### Dilution buffer

- PBS containing 0.5% BSA and 0.05% Tween-20. This buffer can be prepared all at once for the 5 ELISA plates with at least 250 mL PBS under sterile conditions. Add 12.5 ml of BSA stock solution (10%) and 125 µl of Tween-20 to 250 mL PBS, mix gently and store at 4 °C. This solution will be stable for one month when kept sterile.
- For one ELISA plate, 20 mL of Dilution buffer for detection and conjugate solutions, and at least 20 mL for standards and samples (this volume will depend on the number of sample dilutions).



## Human Perforin ELISA Kit

### Coating Antibody

The vial should be stored at 4 °C until December 2021. After reconstitution, the antibodies are stable under sterile conditions at 4 °C for up to 1 year.

- Reconstitute the lyophilized antibody by injecting 250 µL of sterile distilled water into the vial. Mix the solution gently for approximately 15 seconds and allow it to stand for 5 minutes at RT. Avoid vigorous shaking.
- For one ELISA plate: 50 µL is gently but thoroughly mixed with 5 mL PBS.
- **Note:** Do not use commercially available PBS **tablets** for the preparation of the coating solution (the filler in the tablets interferes with the coating process).

### Biotinylated Detection Antibody:

The vial should be stored at 4 °C until October 2021. After reconstitution, the antibodies are stable under sterile conditions at 4 °C for up to 1 year.

- Reconstitute the lyophilized antibody by injecting 500 µL of sterile distilled water into the vial. Mix the solution gently for approximately 15 seconds and allow it to stand for 5 minutes at RT. Avoid vigorous shaking.
- For one ELISA plate: 100 µL is gently and thoroughly mixed with 10 mL Dilution buffer.

### Standard:

The vials should be stored at 4 °C until July 2019.

- Reconstitute the vial with 500 µL of sterile distilled water, gently mix for approximately 15 seconds, and allow to stand for 5 minutes at RT. Avoid vigorous shaking. Thereafter, the reconstituted standard (stock solution) is placed on melting ice and is immediately diluted as indicated below (preferentially within one hour).
- Note: The quantity (expressed in ng/vial) of the Standard is indicated on the vial and is variable for each kit and batch. After reconstitution, the concentration can be calculated as follows: divide the quantity (indicated on the vial) by the volume used for reconstitution. For example, the concentration of a standard containing 5 ng/vial will be 10 ng/mL (= 10,000 pg/mL) after reconstitution in 500µL distilled water.

### SPP Conjugate:

The vial should be stored at -20 °C in the dark until December 2019. After reconstitution, the SPP is stable in the dark under sterile conditions at 4 °C for 2 months.

- Reconstitute the contents of the vial by injecting 500 µL of sterile distilled water into the vial. Mix the solution gently for approximately 15 seconds and allow it to stand for 5 minutes at 4 °C in the dark. Avoid vigorous shaking.



## Human Perforin ELISA Kit

- For one ELISA plate: 100 µL is gently and thoroughly mixed with 10 mL Dilution buffer.

### **TMB substrate solution:**

The vial is ready to use and should be stored at 4 °C until October 2019.

- Bring TMB substrate solution to RT prior to use.

### **BSA Stock Solution:**

The vial should be stored at 4 °C until May 2019. After opening, the reagent is stable under sterile conditions at for up to 6 months.

### **Cytokine Stabilization Buffer:**

The vial should be stored at 4 °C until February 2019. After opening, the reagent is stable under sterile conditions at for up to 6 months.

### **Tween 20:**

The vial should be stored at RT until June 2020.

### **Stop Solution:**

The vial is ready to use and should be stored at 4 °C until August 2019.

- Bring Stop solution to RT prior to use.

### **Sample Preparation**

- Dilute samples in Dilution buffer (at least 1:1). We recommend analyzing a series of dilutions of the sample to ensure that sample measurements fall within the standard curve range (see also section "Interpretation of Results").
- When measuring cytokines in serum or plasma, add 1/20 volume of CSB (ready-to-use) to **pure** serum or plasma sample (CSB is not required for other samples such as cell culture supernatant) before further dilution in Dilution buffer. CSB inhibits the degradation of cytokines.
- It is recommended to analyze samples in triplicate.

### **Preparing the Standards**

With a standard curve, the analyte concentrations in the unknown samples can be determined. The standard curve is generated from the data of 7 two-fold serial dilutions (Std 1-7) of the Standard. The recommended assay range for each specific ELISA kit can be seen in the Specifications Table at the top of the first page. It is recommended to test the standard dilutions (Std 1-7) in triplicate.

- Take 8 tubes and add 400 µL Dilution buffer to 7 of these tubes (Std 2 to Std 7 and Blank).
- Prepare in the remaining tube (Std 1) the highest concentration to be used in the standard curve (see Specifications Table) by mixing an appropriate volume of Standard with Dilution buffer. The final volume of Std 1 should be 800 µL. Allow the mixture to stand for at least 15 seconds before using in further dilutions.

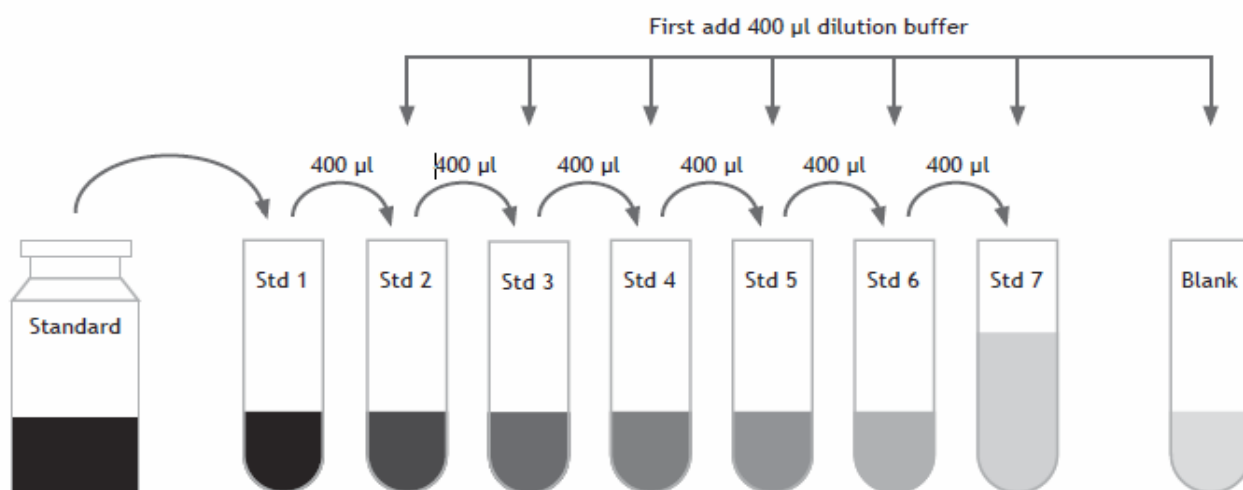


## Human Perforin ELISA Kit

- Perform two-fold serial dilutions: transfer 400  $\mu$ l diluted standard from Std 1 to the next tube (Std 2), mix well and repeat this step until Std 7.

### Notes:

- If less than 10  $\mu$ L of the Standard is needed to make Std 1, it is recommended to dilute the Standard 10 times in Dilution buffer (mix 10  $\mu$ L Standard with 90  $\mu$ L Dilution buffer) and use this to make Std 1.
- A standard curve, including blank, should be run on each ELISA plate.
- Use vials with Standard only once.
- It is recommended to test standard dilutions in triplicate.
- Standard dilutions should be used as soon as possible (preferentially within one hour).



### Directions for washing

- Incomplete washing of the wells will adversely affect the assay. All washing steps should be performed with Wash buffer.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering the tip of an aspiration device into each well (without touching the bottom). After aspiration, fill the wells with at least 250  $\mu$ L Wash buffer and then aspirate the liquid. Repeat these steps at least six times. After washing, the plate is inverted and tapped dry on absorbent tissue paper. Alternatively, the Wash buffer may be put into a squirt bottle. If a squirt bottle is used, empty the wells and flood the plate with Wash buffer, completely filling all wells. Repeat these steps at least six times. After washing, the plate is inverted and tapped dry on absorbent tissue paper.
- When using an automated washing device, follow operating instructions carefully.



## Human Perforin ELISA Kit

### Assay procedure

**Note:** All solutions should reach RT prior to use.

1. Add 50  $\mu$ L of diluted coating antibody solution to each well of the ELISA plate and fill up to 100  $\mu$ L with PBS. Seal the plate to prevent evaporation.
2. Incubate overnight at 4  $^{\circ}$ C (or alternatively 2 hours at 37  $^{\circ}$ C).
3. Remove the coating antibody solution and wash the wells at least 6 times with Wash buffer.
4. Add 200  $\mu$ L of Blocking Buffer to each well.
5. Seal the plate and incubate for 1 hour at 37  $^{\circ}$ C.
6. Remove the Blocking buffer (do not wash the wells).
7. Add 100  $\mu$ L of diluted standards, blank, or samples to each well.
8. Seal the plate and incubate for 2 hours at 37  $^{\circ}$ C (or alternatively overnight at 4  $^{\circ}$ C).
9. Remove standards/samples and wash the wells at least 6 times with Wash buffer.
10. Add 100  $\mu$ L of diluted detector antibody solution to each well.
11. Seal the plate and incubate for 1 hour at 37  $^{\circ}$ C.
12. Remove detection antibody solution and wash the wells at least 6 times with Wash buffer.
13. Add 100  $\mu$ L of diluted SPP conjugate to each well.
14. Seal the plate and incubate for 1 hour at 37  $^{\circ}$ C.
15. Remove SPP conjugate and wash the wells at least 6 times with Wash buffer.
16. Add 100  $\mu$ L of TMB substrate solution into each well.
17. Leave the plate for 20 minutes at RT in the dark. (The substrate produces a soluble end product that is blue in color and can be read at 370 or 655 nm.)
18. After the substrate incubation, do not empty the wells. Stop the reaction by adding 100  $\mu$ L of Stop solution (resulting in a yellow solution) and read the plate at 450 nm within 30 minutes.



## Human Perforin ELISA Kit

### Interpretation of Results

In each experiment, a standard curve needs to be run, consisting of 7 standard dilutions, from which a concentration-response relationship is generated. Construct the standard curve by plotting the mean OD of each standard dilution (Std 1-7) minus the mean Blank (see formula below) on the y-axis against the corresponding concentration on the x-axis.

**Formulas:** Calculated adjusted Standard OD = mean OD of Standard – mean OD of the Blank for each Standard.

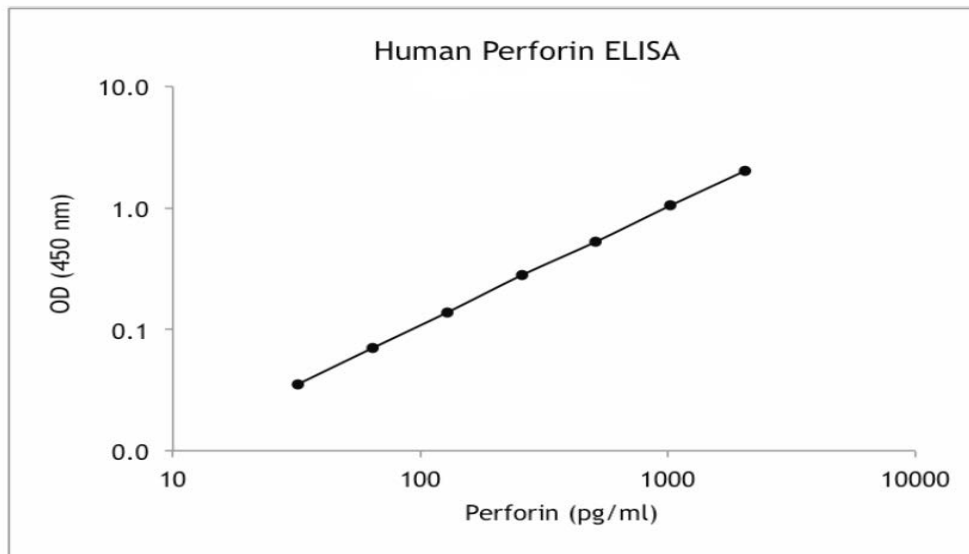
Calculated adjusted Sample OD = mean OD of Sample – mean OD of the Blank for each Sample.

Most laboratories have (plate reader) software that allows various methods of curve fitting. Since ELISA data are essentially sigmoidal rather than linear, we recommend using the 4- or 5-parameter logistic fit for quantitative analysis of the samples. Alternatively, a linear regression curve may be acceptable for the linear portion of the curve consisting of at least 3 concentrations.

After selection of the regression model, the analyte concentrations in unknown samples can be interpolated from the standard curve. The OD value of the sample should fall within the standard curve. Samples showing an OD below the lowest concentration of the standard curve need to be re-analyzed at a lower dilution. Samples showing an OD that exceeds the highest concentration of the standard curve need to be re-analyzed at a higher dilution.

If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

### Typical Data



**Example of a representative Human Perforin standard curve using a 4-parameter logistic fit.**

**NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.**





## Human Perforin ELISA Kit

### Troubleshooting

Problem	Possible cause	Solution
Poor consistency of replicates	Inaccurate pipetting	- Ensure accurate pipetting of volume and avoid air bubbles. - Check pipettes.
	Inadequate mixing of reagents	- Mix reagents adequately.
	Inadequate washing	- Increase the stringency of washes (particularly after the AP conjugate incubation step).
	Too much airbubbles after using a squirt bottle for washing	- You can add a PBS soak step after 5 washing steps with Wash buffer. Use commercially available liquid PBS (pH 7.4).
	Evaporation of solutions	- Ensure precise sealing of the plate.
	Non-homogenous samples or with high particulate matter	- Mix samples thoroughly and remove particulates by centrifugation.
OD <sub>blank</sub> values higher than 0.2	Incubation time of pNPP substrate solution is too long	- Incubation time of substrate should be 20 min.
	Improper storage of pNPP	- Store pNPP at 4 °C and protected from light (unreacted pNPP substrate appears colorless to pale yellow).
	Working solutions were contaminated	- Solutions should be clear and colorless. Use a clean container before addition into wells.
	AP conjugate dilution was too concentrated or left too long on the plate	- Ensure proper dilution of AP conjugate and incubation time.
No signal or low OD values for standards	Improper storage of AP conjugate	- Avoid prolonged exposure to light and heat. - Store conjugate always at 4 °C.
	Incorrect incubation times or temperature	- Ensure proper incubation times. - Reagent solutions should be at RT before use.
	Improper quality or pH of distilled water	- Use distilled water, and not tap water. - Check quality and pH of distilled water.
	Improper standard dilution	- Ensure proper dilution of standard.
	Degradation of antibodies	- Follow recommended storage conditions.
	Overly high washing / aspiration pressure from automated plate washer.	- Check function of washing system or apply manual washing.
Poor standard curve (linearity and dynamic range)	Improper standard dilutions	- Ensure proper dilution of standards (follow 'two-fold dilutions' guidelines).
	Inaccurate pipetting	- Ensure accurate pipetting of volume and avoid air bubbles. - Check pipettes.

