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ab46068
Perforin (PRF1) Human ELISA Kit

For the quantitative measurement of Perforin (PRF1) in supernatants and buffered solutions.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Abcam's Perforin (PRF1) Human in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Perforin in supernatant and buffered solutions.

Note for serum & plasma quantification: high and non-linear detection level is found in Human serum and plasma samples. The high signal appears not dependent to the antibody pairs but probably to the matrix and/or Perforin interaction with other molecules. Consequently, serum and plasma quantification is under the scientist's responsibility and specific investigations. Remark: no detection signal was found in fetal calf serum, rat, mouse and horse serum.

A monoclonal antibody specific for Perforin has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Perforin concentrations, control specimens or unknowns are pipetted into these wells. During the first incubation, the standards or samples and a biotinylated monoclonal antibody specific for Perforin are simultaneously incubated. After washing, the enzyme Streptavidin-HRP, that binds the biotinylated antibody is added, incubated and washed. A TMB substrate solution is added which acts on the bound enzyme to induce a colored reaction product. The intensity of this colored product is directly proportional to the concentration of Perforin present in the samples.

This kit will recognize both endogenous and recombinant Human Perforin.

2. Protocol Summary

Remove appropriate number of antibody coated well strips

⇩

Equilibrate all reagents to room temperature

⇩

Prepare all the reagents, samples, and standards as instructed

⇩

Add standard or sample to each well used. Incubate at room temperature

⇩

Aspirate and wash each well. Add prepared Biotinylated labeled detector antibody. Incubate at room temperature

⇩

Aspirate and wash each well. Add prepared Streptavidin-HRP mix to each well. Incubate at room temperature

⇩

Aspirate and wash each well. Add the TMB Solution to each well until color develops and then add the Stop Solution. Immediately begin recording the color development

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at +4°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity		Storage Condition
	1 x 96 tests	2 x 96 tests	
Perforin Microplate (12 x 8 well strips)	96 wells	2 x 96 wells	+2-8°C
Perforin Standard (Lyophilized)	2 vials	4 vials	+2-8°C
1X Standard Diluent Buffer	15 mL	2 x 25 mL	+2-8°C
Biotinylated anti-Perforin	0.4 mL	2 x 0.4 mL	+2-8°C
Biotinylated Antibody Diluent	7.5 mL	13 mL	+2-8°C
Streptavidin-HRP	2 x 5 µL	4 x 5 µL	+2-8°C
HRP Diluent	12 mL	23 mL	+2-8°C
200X Wash Buffer	10 mL	2 x 10 mL	+2-8°C
Chromogen TMB Substrate Solution	11 mL	24 mL	+2-8°C
Stop Reagent	11 mL	2 x 11 mL	+2-8°C

Note: This ELISA kit will soon contain the "Easy View" colored reagents. The Standard diluent buffer will now be red, and the Streptavidin-HRP Diluent will be green. Please note that while stock lasts you may still receive colorless diluents. This change does not impact the results provided by the kit or the assay procedure.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Tubes to prepare standard or sample dilutions.
- Log-log graph paper or computer and software for ELISA data analysis.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every assay performed.
- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh 1X Wash Buffer. Do not allow wells to sit uncovered or dry for extended periods.
- Kit components should be stored as indicated. All the reagents should be equilibrated to room temperature before use. Reconstituted standards should be discarded after use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remaining strips from degradation.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross-contamination; for the dispensing of the Stop Solution and substrate solution, avoid pipettes with metal parts.
- Thoroughly mix the reagents and samples before use by agitation or swirling.
- All residual washing liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells.
- The TMB solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB solution with metal to prevent color development. Warning TMB is toxic avoid direct contact with hands. Dispose of properly.

- If a dark blue color develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbances within 1 hour after completion of the assay.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.
- Dispense the TMB solution within 15 minutes following the washing of the microtiter plate.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Wash Buffer

Dilute the 200X Wash Buffer Concentrate 200-fold in distilled water before use. Mix gently to avoid foaming.

The 1X Wash Buffer can be prepared as needed according to the following table:

Number of well strips used	Volume of 200X Wash Buffer Concentrate (mL)	Volume of distilled water (mL)
1-6	5	995
1-12	10	1,990

9.2 1X Biotinylated anti-Perforin

Prepare the 1X Biotinylated anti-Perforin immediately prior to use. According to the table below, dilute the Biotinylated anti-Perforin with the Biotinylated Antibody Diluent based on the number of wells being used in the assay procedure:

Number of well strips used	Volume of Biotinylated anti-Perforin (µL)	Volume of Biotinylated Antibody Diluent (µL)
2	40	1,060
3	60	1,590
4	80	2,120
6	120	3,180
12	240	6,360

9.3 1X Streptavidin-HRP Solution

Add 500 µL of HRP-Diluent to the Streptavidin-HRP vial prior to use to create a Streptavidin-HRP Concentrate. Do not keep this solution for further experiments.

Subsequently, prior to use in the assay procedure, prepare the 1X Streptavidin-HRP Solution by further diluting the Streptavidin-HRP Concentrate with HRP-Diluent. Use the table below to determine the volumes of each solution required to prepare the final 1X Streptavidin-HRP Solution:

Number of well strips used	Volume of Streptavidin HRP (µL)	Volume of HRP Diluent (mL)
2	30	2
3	45	3
4	60	4
6	75	5
12	150	10

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Prepare a 2,000 pg/mL **Standard #1** by reconstituting lyophilized Perforin standard with the volume indicated on the vial using the 1X Standard Diluent Buffer.

10.2 Label tubes #2-6 and add 100 µL of 1X Standard Diluent Buffer into each tube.

10.3 Prepare **Standard #2** by adding 100 µL of Standard #1 to tube #2 and mix thoroughly.

10.4 Prepare **Standard #3** by adding 100 µL of Standard #2 to tube #3 and mix thoroughly.

10.5 Using the table below as a guide, prepare further serial dilutions.

10.6 1X Standard Diluent Buffer serves as the zero standard (0 pg/mL).

Standard #	Volume to dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting conc. (pg/mL)	Final conc. (pg/mL)
1	-	-	-	2,000	2,000
2	100	100	200	2,000	1,000
3	100	100	200	1,000	500
4	100	100	200	500	250
5	100	100	200	250	125
6	100	100	200	125	62.5

11. Sample Preparation

11.1 Preparation of Cell culture Supernatants:

Centrifuge cell culture media at 1,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

12. Plate Preparation

- The 96 well plate strips included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prior to use, mix all reagents thoroughly taking care not to create any foam within the vials.

13.2 Determine the number of microplate strips required to test the desired number of samples, plus appropriate number of wells needed for controls and standards. Remove sufficient microplate strips from the pouch.

13.3 Add 100 µL of each standard (see Section 10), including blank controls to the appropriate wells.

13.4 Add 100 µL of sample to the appropriate wells.

13.5 Cover and incubate for 1 hour at room temperature (18-25°C).

13.6 Remove the cover and wash the plate as follows:

13.6.1 Aspirate the liquid from each well.

13.6.2 Add 0.3mL of 1X Wash Buffer into each well.

13.6.3 Aspirate the liquid from each well.

13.6.4 Repeat for a total of 3 washes.

13.7 Add 50 µL of 1X Biotinylated anti-Perforin to all wells (see Section 9).

13.8 Cover and incubate for 1 hour at room temperature (18-25°C). Repeat wash step 13.6.

13.9 Add 100 µL of 1X Streptavidin-HRP solution into all wells, including the blank wells. Re-cover and incubate at room temperature for 30 minutes.

13.10 Wash as described in Step 13.6.

13.11 13.11 Add 100 µL of Chromogen TMB substrate solution into each well and incubate in the dark for 10-20 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil.

Δ Note*:* Incubation time of the substrate solution is usually determined by the microplate reader performances: many microplate readers record absorbance only up to 2.0 O.D. The O.D. values of the plate should be monitored and the substrate reaction stopped before positive wells are no longer accurately readable (maximum ~20 minutes).

13.12 Add 100 µL of Stop Reagent into each well. Results must be taken immediately after the addition of Stop Reagent, or within one hour, if the microplate is stored at 2-8°C in the dark.

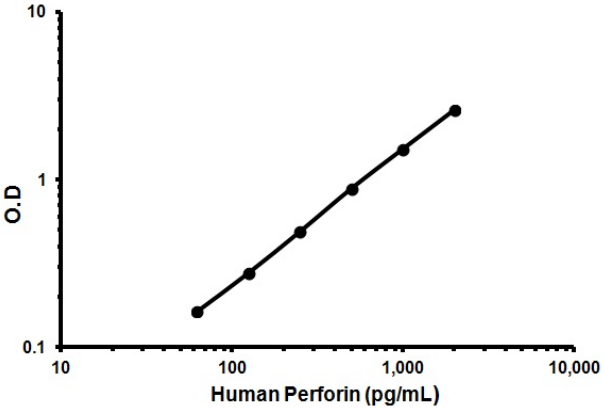
13.13 Read absorbance of each well on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm (610 nm to 650 nm is acceptable) as the reference wavelength.

14. Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D.
2,000	2.579
1,000	1.513
500	0.882
250	0.489
125	0.278
62.5	0.164
0	0.070

Figure 1. Example of Human Perforin standard curve.

16. Typical Sample Values

SENSITIVITY –

The minimum detectable dose of Perforin is <40 pg/mL. This has been determined by adding 3 standard deviations to the mean optical density obtained when the zero standard was assayed 40 times.

High and non-linear detection level is found in Human serum and plasma samples. The high signal appears not dependent to the antibody pairs but probably to the matrix and/or Perforin interaction with other molecules. Consequently, serum and plasma quantification is under the scientist's responsibility and specific investigations.

PRECISION – Sample A

	Intra-assay Precision	Inter-Assay Precision
n=	8	9
Mean (ng/mL)	416	580
SD	20	15
CV (%)	5	3

PRECISION – Sample B

	Intra-assay Precision	Inter-Assay Precision
n=	8	9
Mean (ng/mL)	674	665
SD	25	31
CV (%)	4	5

PRECISION – Sample B

	Intra-assay Precision	Inter-Assay Precision
n=	8	9
Mean (ng/mL)	984	1,004
SD	24	45
CV (%)	3	5

DILUTION PARALLELISM –

Three stimulated cellular supernatant with different levels of Perforin were analysed at different serial two fold dilutions with two replicates each. The linearity between expected and measured concentrations is equal to 0.99.

17. Assay Specificity

This assay recognizes natural Human Perforin. To define specificity of this ELISA, several proteins were tested for cross reactivity. There was no cross reactivity observed for any protein tested (Granzyme B, Fas, Fas L, IL-1β, IL-2, IFNγ, TNFδ, TRAIL, TRAIL R3, TRAIL R4)

No detection signal was found in fetal calf serum, rat, mouse and horse serum.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Low Precision / Large CV	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique
	Contaminated wash buffer	Prepare fresh wash buffer
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner

Low Precision / Large CV	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)