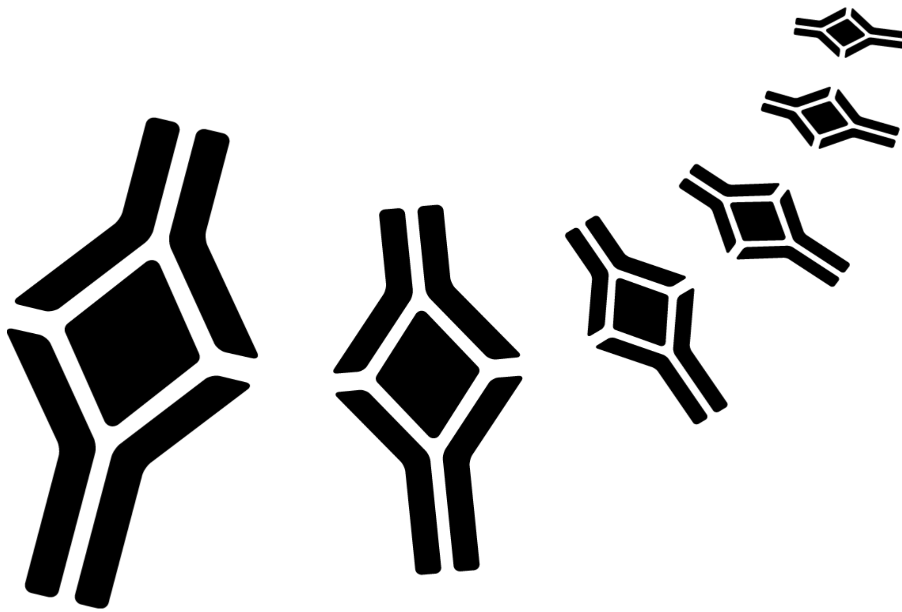

HUMAN PERFORIN ELISA

Product Data Sheet

Cat. No.: RGP021R

For Research Use Only

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This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.

Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The BioVendor Human Perforin ELISA is to be used for the in-vitro quantitative determination of Perforin in supernatant, buffered solutions. The assay will recognize natural Perforin.

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.

This kit has been configured for research use only.

2. PRINCIPLE OF THE METHOD

The Perforin Kit is a solid phase sandwich Enzyme Linked-Immuno- Sorbent Assay (ELISA). A monoclonal antibody specific for Perforin has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Perforin concentrations and unknowns are pipetted into these wells.
During the first incubation, the Perforin antigen is added to wells. After washing,a biotinylated monoclonal antibody specific for Perforin is incubated. Then the enzyme (streptavidin-peroxydase) is added. After incubation and washing to remove all unbound enzyme, a substrate solution which acts on the bound enzyme is added to induce a coloured reaction product. The intensity of this coloured product is directly proportional to the concentration of Perforin present in the samples.

3. REAGENTS PROVIDED AND RECONSTITUTION

REAGENTS (Store at 2-8°C)	**Quantity**	**State**
Antibody Coated Microtiter Strips	96 wells	Ready to use (Pre-coated)
Plastic plate covers	2	n/a
Standard: 2000 pg/ml	2 vials	Reconstitute as directed in Quality Control Sheet (see reagent preparation)
Standard Diluent (Buffer)	1 vial (25 ml)	Ready to use
Biotinylated anti-Perforin	1 vial (0.4 ml)	Dilute in Biotinylated antibody diluent (see reagent preparation)
Biotinylated Antibody diluent	1 vial (7 ml)	Ready to use
Streptavidin-HRP	2 vials (5µl)	Add 0.5ml of HRP diluent prior to use (see reagent preparation)
HRP Diluent	1 vial (23 ml)	Ready to use
Wash Buffer	1 vial (10 ml)	200x Concentrate dilute in distilled water (see reagent preparation)
TMB Substrate:	1 vial (11 ml)	Ready to use
H_2SO_4 : Stop Reagent	1 vial (11 ml)	Ready to use

4. MATERIAL REQUIRED BUT NOT PROVIDED

- Microtitre plate reader fitted with appropriate filters (450 nm required with optional 620 nm reference filter)
- Microplate washer or wash bottle
- 10, 50, 100, 200 and 1,000 µl adjustable single channel micropipettes with disposable tips
- 50-300 µl multi-channel micropipette with disposable tips
- Multichannel micropipette reagent reservoirs
- Distilled water
- Vortex mixer
- Miscellaneous laboratory plastic and/or glass, if possible sterile

5. STORAGE INSTRUCTIONS

Store kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C). Expiry of the kit and reagents is stated on box front labels. The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

Wash Buffer: Once prepared store at 2-8°C for up to 1 week
Standards : Once prepared use immediately and do not store
Biotinylated Secondary Antibody: Once prepared use immediately and do not store
Streptavidin-HRP: Once prepared use immediately and do not store

6. SPECIMEN COLLECTION, PROCESSING & STORAGE

Cell culture supernatants, serum, plasma or other biological samples will be suitable for use in the assay. Remove serum from the clot or red cells, respectively, as soon as possible after clotting and separation.

Cell culture supernatants: Remove particulates and aggregates by spinning at approximately 1000 x g for 10 min.

Storage: If not analyzed shortly after collection, samples should be aliquoted (250-500µl) to avoid repeated freeze-thaw cycles and stored frozen at –70°C. Avoid multiple freeze-thaw cycles of frozen specimens.

Recommendation: Do not thaw by heating at 37°C or 56°C. Thaw at room temperature and make sure that sample is completely thawed and homogeneous before use. When possible avoid use of badly haemolysed or lipemic sera. If large amounts of particles are present these should be removed prior to use by centrifugation or filtration.

7. SAFETY & PRECAUTIONS FOR USE

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures , e.g.CDC/NIH Health manual : " Biosafety in Microbiological and Biomedical Laboratories" 1984
- Laboratory gloves should be worn at all times
- Avoid any skin contact with H_2SO_4 and TMB. In case of contact, wash thoroughly with water
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used
- Do not pipette by mouth
- When not in use, kit components should be stored refrigerated or frozen as indicated on vials or bottles labels
- All reagents should be warmed to room temperature before use. Lyophilized 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 deterioration
- Cover or cap all reagents when not in use
- Do not mix or interchange reagents between different lots
- Do not use reagents beyond the expiration date of the kit
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of H_2SO_4 and substrate solution, avoid pipettes with metal parts
- Use a clean plastic container to prepare the washing solution
- 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 colour development. Warning TMB is toxic avoid direct contact with hands. Dispose off properly
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded. Read absorbance's 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
- Follow incubation times described in the assay procedure
- Dispense the TMB solution within 15 min of the washing of the microtitre plate

8. ASSAY PREPARATION

Bring all reagents to room temperature before use

8.1 Assay Design

Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running zeros and standards. Each sample, standard and zero should be tested **in duplicate**. Remove sufficient Microwell Strips for testing from the pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly and return to 2-8°C storage.

Example plate layout (example shown for a 6 point standard curve)

	Standards / Controls		Sample wells									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	2000	2000										
B	1000	1000										
C	500	500										
D	250	250										
E	125	125										
F	62.5	62.5										
G	Blank	Blank										
H												

All remaining empty wells can be used to test samples in duplicate

8.2. Preparation of Wash Buffer

Dilute the (200x) wash buffer concentrate 200 fold with distilled water to give a 1x working solution. Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder. Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming. Transfer to a clean wash bottle and store at 2˚-8˚C for up to 1 week.

8.3. Human serum and plasma samples

See special note on page 3 of this product data sheet.

8.4. Preparation of Standard

Standard vials must be reconstituted with the volume of Standard Diluent shown on the vial immediately prior to use. This reconstitution gives a stock solution of 2000 pg/ml of Perforin. Mix the reconstituted standard gently by inversion only. Serial dilutions of the standard are made directly in the assay plate to provide the concentration range from 2000 to 62.5pg/ml. A fresh standard curve should be produced for each new assay.

- Immediately after reconstitution add 200µl of the reconstituted standard to wells A1 and A2, which provides the highest concentration standard at 2000pg/ml
- Add 100µl of Standard Diluent to the remaining standard wells B1 and B2 to F1 and F2
- Transfer 100µl from wells A1 and A2 to B1 and B2. Mix the well contents by repeated aspirations and ejections taking care not to scratch the inner surface of the wells
- Continue this 1:1 dilution using 100µl from wells B1 and B2 through to wells F1 and F2 providing a serial diluted standard curve ranging from 2000pg/ml to 62.5pg/ml
- Discard 100µl from the final wells of the standard curve (F1 and F2)

Alternatively these dilutions can be performed in separate clean tubes and immediately transferred directly into the relevant wells.

8.5. Preparation of Biotinylated anti-Perforin

It is recommended this reagent is prepared immediately before use. Dilute the biotinylated anti-Perforin with the biotinylated antibody diluent in an appropriate clean glass vial using volumes appropriate to the number of required wells. Please see example volumes below:

Number of Wells used	Biotinylated Antibody (µl)	Biotinylated Antibody Diluent (µl)
16	40	1060
24	60	1590
32	80	2120
48	120	3180
96	240	6360

8.6. Preparation of Streptavidin-HRP

It is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.

Dilute the 5 µl vial with 0.5 ml of HRP diluent **immediately before use.** Do-not keep this diluted vial for future experiments. Further dilute the HRP solution to volumes appropriate for the number of required wells in a clean glass vial. Please see example volumes below:

Number of Wells	Streptavidin-HRP(µl)	Strep-HRP Diluent (ml)
16	30	2
24	45	3
32	60	4
48	75	5
96	150	10

9. METHOD

We strongly recommend that every vial is mixed thoroughly without foaming prior to use except the standard vial which must be mixed gently by inversion only.
Prepare all reagents as shown in section 8.
Note: Final preparation of Biotinylated anti-Perforin (section 8.5) and Streptavidin-HRP (section 8.6) should occur immediately before use.

Assay Step		Details
1.	Addition	**Prepare Standard curve** as shown in section 8.4
2.	Addition	Add 100µl of each **sample and standard** in duplicate to appropriate number of wells
3.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour**
4.	Wash	Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.3 ml of **1x washing solution** into each well c) Aspirate the contents of each well d) Repeat step b and c another two times
5.	Addition	Add 50µl of diluted **biotinylated anti-Perforin** to all wells
6.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **1 hour**
7.	Wash	Repeat wash step 4.
8.	Addition	Add 100µl of **Streptavidin-HRP** solution into all wells
9.	Incubation	Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for **30 min.**
10.	Wash	Repeat wash step 4.
11.	Addition	Add 100µl of ready-to-use **TMB Substrate Solution** into all wells
12.	Incubation	Incubate in the dark for **10-20 minutes*** at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil
13.	Addition	Add 100µl of **H_2SO_4:Stop Reagent** into all wells
Read the absorbance value of each well (immediately after step 13.) on a spectrophotometer using 450 nm as the primary wavelength and optionally 630 nm as the reference wave length (610 nm to 650 nm is acceptable).		

*_Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range_

10. DATA ANALYSIS

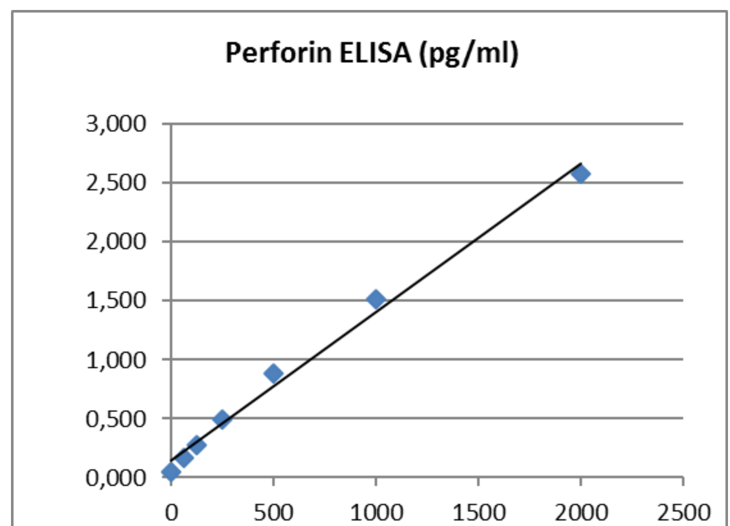
Calculate the average absorbance values for each set of duplicate standards and samples. Ideally duplicates should be within 20% of the mean.

Generate a linear standard curve by plotting the average absorbance of each standard on the vertical axis versus the corresponding human Perforin standard concentration on the horizontal axis.

The amount of Perforin in each sample is determined by extrapolating OD values against Perforin standard concentrations using the standard curve.

Example Perforin Standard curve

Standard	Perforin Conc.	OD (450nm) mean	CV (%)
1	2000	2.579	0.2
2	1000	1.513	7.5
3	500	0.882	5.3
4	250	0.489	4.0
5	125	0.278	2.3
6	62.5	0.164	0.4
Zero	0	0.052	-



Note: curve shown above should not be used to determine results. Every laboratory must produce a standard curve for each set of microwell strips assayed.

11. ASSAY LIMITATIONS

Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with Standard diluent or with your own sample buffer to produce an OD value within the range of the standard curve. Following analysis of such samples always multiply results by the appropriate dilution factor to produce actual final concentration.

The influence of various drugs and aberrant sera (hemolyzed, hyperlipidemic, jaundiced...) on end results has not been investigated. Bacterial or fungal contamination and laboratory cross-contamination may also cause irregular results. The rate of degradation of native Perforin in various matrices has not been investigated.

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 Washing Buffer, fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.

As with most biological assays conditions may vary from assay to assay therefore **a fresh standard curve must be prepared and run for every assay.**

12. PERFORMANCE CHARACTERISTICS

12.1 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.

12.2 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, TNFα, TRAIL, TRAIL R3, TRAIL R4)

12.3 Precision

Intra-assay

Sample	n	Mean (pg/mL)	SD	CV%
A	8	416	20	5
B	8	674	25	4
C	8	984	24	3

Inter-assay

Sample	n	Mean (pg/mL)	SD	CV%
A	9	580	15	3
B	9	665	31	5
C	9	1004	45	5

12.4 Dilution Linearity

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.**

Sample	Dilution	Expected values pg/ml	Measured values pg/ml	% recovery of expected values	Linearity R^2 expected / measured
D	1/2	333	333	100	0,9963
	1/4	167	175	105	
	1/8	83	75	90	
E	1/2	1014	1014	100	0,9998
	1/4	507	576	114	
	1/8	254	346	136	
F	1/2	708	708	100	0,9999
	1/4	354	382	108	
	1/8	177	223	126	

13. REFERENCES

Aydemir, T. B. et al. Zinc transporter ZIP8 (SLC39A8) and zinc influence IFN-{gamma} expression in activated human T cells. J. Leukoc. Biol.,2009; jlb.1208759

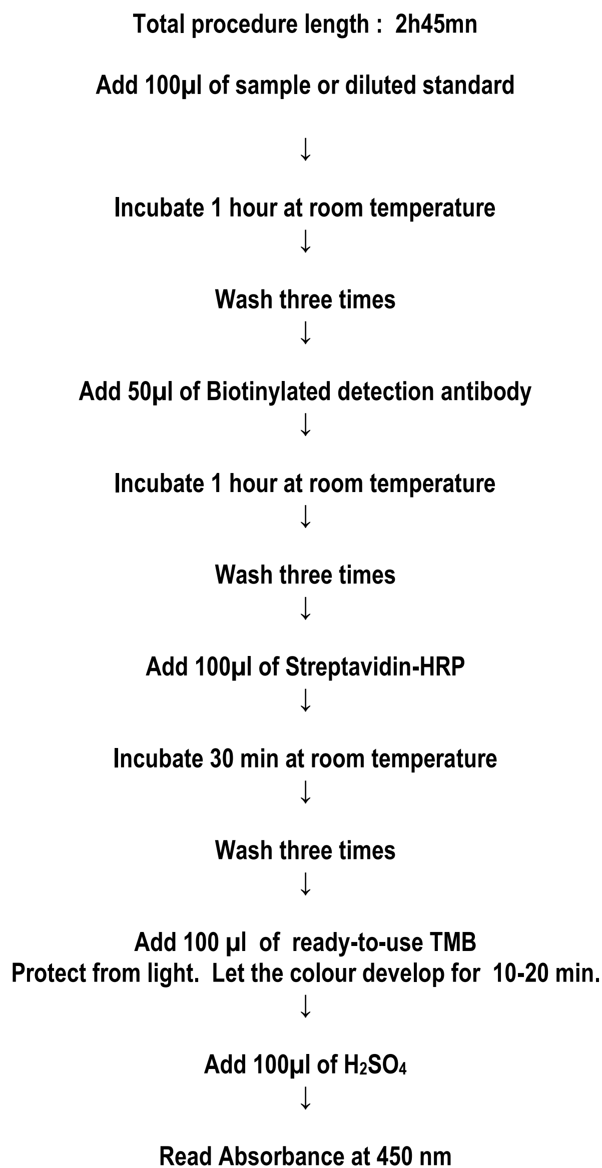
Chiossone, L. et al. Molecular analysis of the methylprednisolone-mediated inhibition of NK cell function: evidence for different susceptibility of IL-2-versus IL-15-activated NK cells. Blood,2007; 109: 3767 – 3775

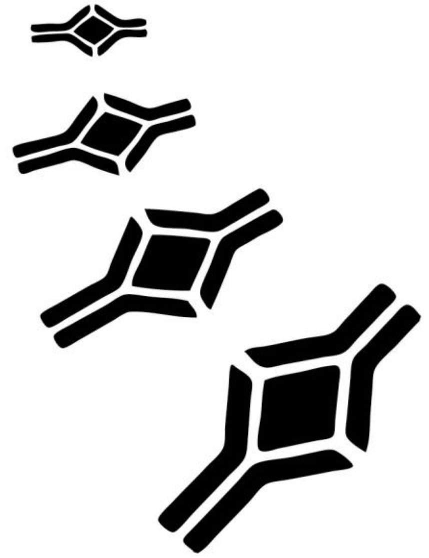
Lagadari, M., et al. Lysophosphatidic acid inhibits the cytotoxic activity of NK cells: involvement of Gs protein-mediated signaling. Int. Immunol.,2009; 21(6): 667-677

Schmidt, S. et al. Human Natural Killer Cells Exhibit Direct Activity against Aspergillus fumigatus Hyphae, But Not Against Resting Conidia. The Journal of Infectious Disease, 2010

Stary, G. et al. Tumoricidal activity of TLR7/8-activated inflammatory dendritic cells. J Exp Med.,2007;(204): 1441-1451

14. ASSAY SUMMARY





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