PEPROTECH
OUR SUPPORT, YOUR DISCOVERY

biogems
A PeproTech Brand

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NEOBIOSCIENCE

ELISA Products

About PeproTech

PeproTech creates the building blocks of your life science research by manufacturing high-quality recombinant cytokines and related reagents that advance scientific discovery and human health.

Since 1988, we have grown into a global enterprise with state-of-the-art manufacturing facilities in the US, and offices around the world. With over 2,000 products, PeproTech has developed and refined innovative protocols to ensure quality, reliability and consistency.

Our mission is to provide the highest quality products and premium support that address the needs and demands of today's scientists and researchers. We pride ourselves on being a trusted partner within the scientific community.

Our products:

- Comprehensive line of Cytokines and Antibodies
- GMP Cytokines for Cell, Gene and Tissue Therapy
- Animal-Free Cytokines
- ELISA Development Kits
- Cell Culture Media Kits / Supplements

High Quality

Tens of thousands of references will speak for themselves

Competitive Pricing

Ask for a quote and get the best value for your money

Premium Technical Support

Contact our experts by phone or email

ELISA Product Lines Offered

BioGems Ready to Use ELISA Kits

Pre-coated plates

Ready to Use Kits contain all the components, including buffers, required for the quantitative measurement of proteins from any sample source.
Assay time: about 205 min (37°C) - 280 min (RT).

Simplify your test and expedite your experiment by allowing us to do the preparation steps.

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PeproTech ELISA Development Kits (EDK)

Plate-coating is required - optimized protocol provided

EDKs contain the key components required for the quantitative measurement of proteins from any sample source. The complementary required components/ buffers can be purchased separately.
Assay time: Overnight + 350 - 380 minutes.

For the Scientist with ELISA experience looking to manage a budget wisely.

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PeproTech ELISA Components

Construct your own ELISA with PeproTech's Antigen Affinity Purified Antibodies, Biotinylated Antibodies and Recombinant Protein Standards for the quantitative measurement of proteins from any sample source. Complementary components/buffers can be purchased separately.
Assay time: Overnight + 350 - 380 minutes.

End-user testing, and optimization is required. The compatibility of these components with the ELISA format may or may not have been verified by PeproTech.

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PeproTech ELISA Buffer Kit

A supplemental buffer kit containing all of the additional components/buffers required to run PeproTech's EDKs or optimize your own ELISA.

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An Introduction to ELISA

The ELISA is designed to quantitatively or qualitatively detect a specific analyte in a sample. It can be applied in various formats according to the nature of the analyte. The first thing you have to decide is which ELISA format is the best for your needs.

Direct ELISA - The direct ELISA is the simplest ELISA format. In a direct ELISA, the analyte in the sample is immobilized on a solid support (most commonly a 96-well microtiter plate) and detected directly by an antibody conjugated to an enzyme. Alternatively, but less common antibodies in the sample are immobilized onto the plate and are directly detected by enzyme labeled antigen. The main advantages of direct ELISA are that it is faster and less prone to error since fewer steps are required. However, it is not suitable for complex biological samples. It is commonly used for titrating conjugated secondary antibodies and estimating antigen cross-reactivity.

Indirect ELISA - The indirect ELISA is a variation of direct ELISA. In an indirect ELISA, the antigen is immobilized on a solid support followed by an unconjugated, antigen-specific antibody that binds to the target antigen. Subsequently, an enzyme-conjugated secondary antibody binds to the primary antibody. The final reaction produces signal, which is proportional to the amount of the bound primary antibody. Indirect ELISA is more sensitive than direct ELISA and offers greater flexibility since different primary antibodies can be detected by a single, labeled secondary antibody. Indirect ELISA is the method of choice for determining the titer of target-specific antibodies in samples such as patients' serum, anti-serum or hybridoma supernatants.

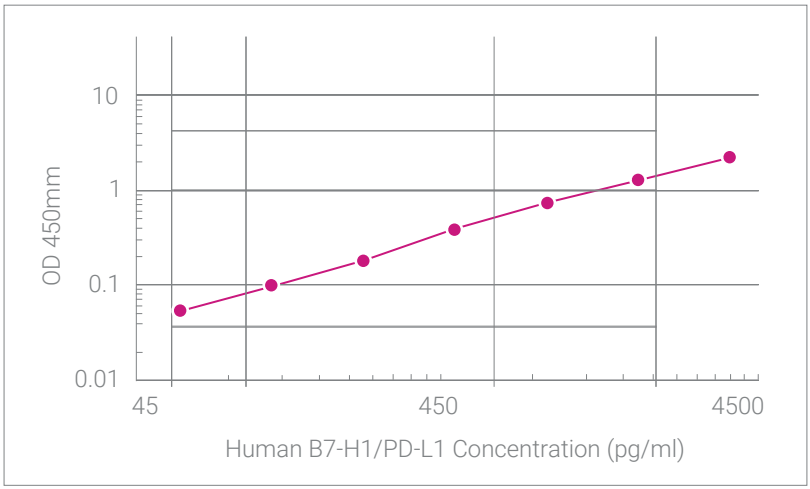
Sandwich ELISA - Sandwich ELISA is the most common commercially available ELISA format. This format requires the use of matched antibody pairs or polyclonal antibodies that can bind different, non-overlapping epitopes on the target antigen. A capture antibody is immobilized onto the wells of the microplate. This antibody captures the target analyte from the sample and the bound target antigen is then detected by a secondary detection antibody. If the detection antibody is conjugated to an enzyme, then the assay is called a direct sandwich ELISA. If the detection antibody is unlabeled, then a second, enzyme-labeled detection antibody is needed, resulting in an indirect sandwich ELISA. This format is highly specific due to the use of two antigen specific antibodies, it is suitable for complex samples and flexible in the detection method. Sandwich ELISA is the ideal format to quantify soluble antigens.

Competitive ELISA - In the competitive format the signal is reciprocal to the target concentration in the sample, i.e. as antigen concentration in a sample increases, signal intensity decreases. The capture antibody is immobilized to the solid matrix followed by incubation with the sample containing the target. The signal is then obtained by adding labeled antigen. The antigen in the sample binds to the capture antibody reducing the amount of the labeled antigen that can bind to the capture antibody. Alternatively, the antigen is immobilized on the plate and the sample containing the antigen is incubated with labeled detection antibody. The antigen in the sample binds to the detection antibody and reduces the amount of the detection antibody that can bind to the immobilized antigen. Competitive ELISA is primarily used when only one antibody is available to the antigen of interest or when the analyte is small, e.g. a hapten, and cannot bind two different antibodies.

ALL PEPROTECH ELISA KITS FOLLOW THE SANDWICH ELISA FORMAT

An Introduction to ELISA

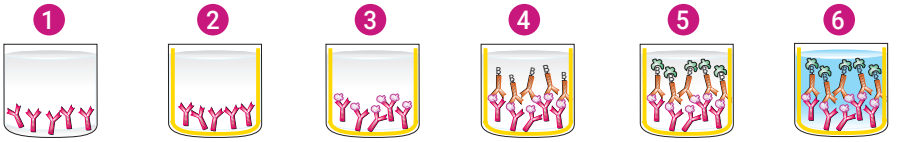
Typical standard curve - Human B7-H1/PD-L1 Pre-Coated ELISA Kit



Standard #	8	7	6	5	4	3	2	1
Standard concentration (pg/ml)	0	62.5	125	250	500	1000	2000	4000
OD_{450}	0.003	0.055	0.101	0.183	0.388	0.744	1.272	2.194

An Introduction to Sandwich ELISA

Schematic representation of a Sandwich ELISA Protocol



Coating with antigen-specific capture antibody

Blockage of non-specific binding sites

Incubation with the biotinylated detection Ab. Capture-Ab-Antigen-Detection Ab Complex formation

Incubation with the biotinylated detection Ab. Capture-Antigen-Detection Complex formation

Addition of HRP-linked Streptavidin/ Avidin which binds to the complex

Addition of substrate. Colorless substrate is converted into a soluble colored solution

1.
Prepare antigen-specific antibody (capture antibody) solution in coating buffer. Add the antibody solution to each well and incubate. When incubation time is complete, wash away unbound antibody.

2.
Add blocking buffer and incubate. When incubation is over, wash away the blocking agent.

* Inefficient blocking of non-specific binding sites may lead to high background noise. If this is observed, increase the incubation time or try other blocking agent.

3.
Add freshly prepared solutions of standards, samples or controls and incubate. When incubation is over, wash the wells several times.

4.
Add biotinylated detection antibody solution and incubate. When incubation is over, wash the wells several times.

5.
Add Streptavidin/Avidin-peroxidase conjugate solution and incubate. When incubation is over, wash the wells several times.

6.
Add TMB/ABTS substrate solution and incubate. After addition of stop solution, read optical density using microplate reader.

BioGems Ready to Use ELISA Kits (Pre-coated & Blocked)

Kit contents:

- Target specific antibody Pre-coated 96-well strip microplate (12 strips of 8 wells) pre-coated with target-specific antibody and blocking buffer
- Lyophilized Target Protein Standard
- Biotinylated Detection Antibody Solution
- Avidin-Biotin-Peroxidase Complex Solution
- Sample Diluent Solution
- Antibody Diluent Solution
- Avidin-Biotin-Peroxidase Diluent Solution
- Wash Buffer Concentrate
- TMB Color Developing Reagent
- Stop Solution
- Plate Sealer Films

Assay procedure summary

Coated Microplate

Standard/sample incubation	**1st reaction 120 minutes**
Washing	
Detection antibody incubation	**2nd reaction 90 minutes**
Washing	
Avidin-Biotin-Peroxidase Complex incubation	**3rd reaction 40 minutes**
Washing	
TMB Color Developing Reagent incubation	**Color reaction 30 minutes**
Stop solution	Important: read the results within **30 minutes** of stopping the reaction

Note: Shorter incubation times are recommended when performing incubations at 37°C.

BioGems Pre-coated Kits

Name	Species	Cat #	Price
April	Human	BGK2QBA2	$410
ANG-2	Human	BGK15123	$440
B7-1	Human	BGK33681	$435
PD-L1/B7-H1	Human	BGK9NZQ7	$410
BDNF	Human	BGK23560	$380
BMP-2	Human	BGK8C060	$425
CD40 Ligand	Human	BGK29965	$490
	Murine	BGK27548	$435
EGF	Human	BGK01133	$360
Epiregulin	Human	BGK14944	$420
FAS	Human	BGK25445	$370
FLT-3 Ligand	Human	BGK49771	$405
FSTL1	Human	BGK12841	$475
Galectin-3	Human	BGK17931	$455
GM-CSF	Rat	BGK48750	$445
Growth Hormone	Human	BGK01241	$435
HB-EGF	Human	BGK99075	$415
HGF	Human	BGK14210	$370
	Murine	BGK08048	$410
IFN-γ	Human	BGK01579	$345
	Murine	BGK01580	$340
IGF-1	Murine	BGK9PU89	$400
IL-10	Human	BGK22301	$345
	Murine	BGK18893	$320
IL-12 (p70)	Human	BGK29459	$345
	Murine	BGK43431	$320
IL-13	Human	BGK35225	$390
	Murine	BGK20109	$485
	Rat	BGK42203	$450
IL-17A	Human	BGK16552	$345
	Murine	BGK62386	$340
IL-17E/IL-25	Human	BGK9H293	$465
IL-1α	Human	BGK01583	$350

BioGems Pre-coated Kits

Name	Species	Cat #	Price
IL-1α	Rat	BGK16598	$450
IL-1β	Human	BGK01584	$345
	Murine	BGK10749	$340
	Rat	BGK5BKB0	$450
IL-2	Human	BGK60568	$345
	Murine	BGK04351	$295
IL-22	Human	BGK9GZX6	$400
IL-3	Human	BGK08700	$350
	Murine	BGK01586	$405
IL-4	Human	BGK05112	$345
	Murine	BGK07750	$345
	Rat	BGK20096	$395
IL-6	Human	BGK05231	$345
	Murine	BGK08505	$305
	Rat	BGK20607	$445
IL-7	Human	BGK8K673	$405
	Murine	BGK10168	$440
IL-8	Human	BGK10145	$345
JE/MCP-1	Murine	BGK10148	$435
KC (CXCL1)	Murine	BGK2RTH0	$435
LIGHT	Human	BGK43557	$420
	Murine	BGK9QYH9	$400
LIX (CXCL6)	Murine	BGK50228	$425
MCP-1 (CCL2)	Human	BGK13500	$370
M-CSF	Human	BGK09603	$380
MIF	Human	BGK4AY87	$465
MIP-3α (CCL20)	Human	BGK78556	$445
MIP-3β (CCL19)	Human	BGK6IBD6	$445
	Murine	BGK70460	$395
Neuregulin	Human	BGK02297	$415
RANTES	Human	BGK0EI67	$420
sRANK Ligand	Murine	BGK35235	$445
sTNF Receptor	Human	BGK20333	$410

BioGems Pre-coated Kits

Name	Species	Cat #	Price
TGF-α	Human	BGK01135	$435
TGF-β1	Human	BGK01137	$410
	Murine	BGK04202	$400
TGF-β3	Human	BGK10600	$440
TLR-3	Human	BGK6Y0F1	$460
TNF-α	Human	BGK01375	$345
	Murine	BGK06804	$305
	Rat	BGK16599	$445
VEGF	Human	BGK15692	$365
	Murine	BGK00731	$400
β-NGF	Murine	BGK01139	$375

PeproTech ELISA Development Kits (EDK)

Each ELISA Development Kit contains the following components (Depicted in Red in the diagram): Capture antibody, Detection antibody (biotinylated), a calibrated protein Standard, and Avidin/Strepavidin-HRP Conjugate. The optimal concentration of capture antibody and detection antibody is provided with each kit. Using the suggested concentrations will provide the highest sensitivity combined with the least amount of background. Quantities are sufficient to detect the target cytokine in at least 1,000 ELISA plate wells. (Mini kits are sufficient to run at least 200 ELISA plate wells.)

Additional components (Grey) necessary to run an ELISA are not supplied, but can be purchased as a separate kit. ELISA Buffer Kit-Catalog # 900-K00/900-T00 (see page 18).

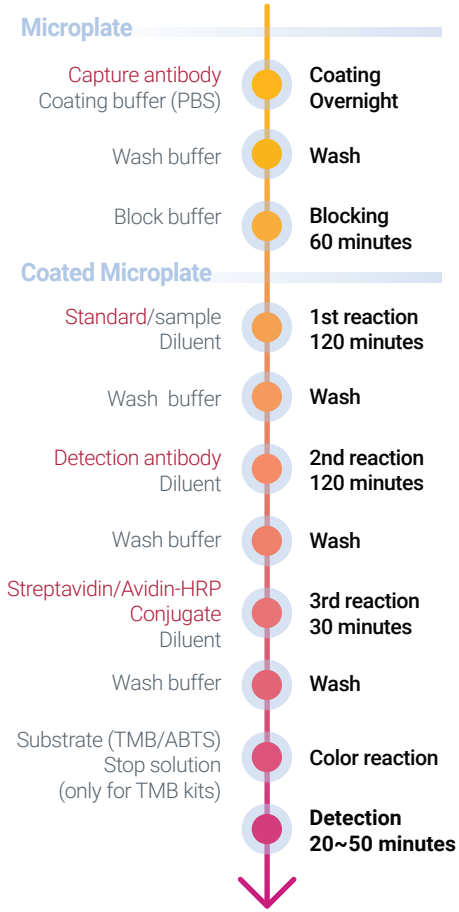
Kit contents:

- Protein Standard
- Capture Antibody
- Detection Antibody
- Streptavidin/Avidin-HRP Conjugate

Required components (Not supplied)

- Uncoated Microplates
- Plate Coating Buffer
- Wash Buffer
- Blocking Buffer
- Sample Diluent
- Substrate (ABTS/TMB)
- Sealing Films
- Stop solution (for TMB kits)

For ELISA Buffer Kit



* The ELISA Buffer kit contains all of the necessary components (Grey) to assay ten ELISA plates. Refer to next page.*

PeproTech ELISA Development Kits (EDK)

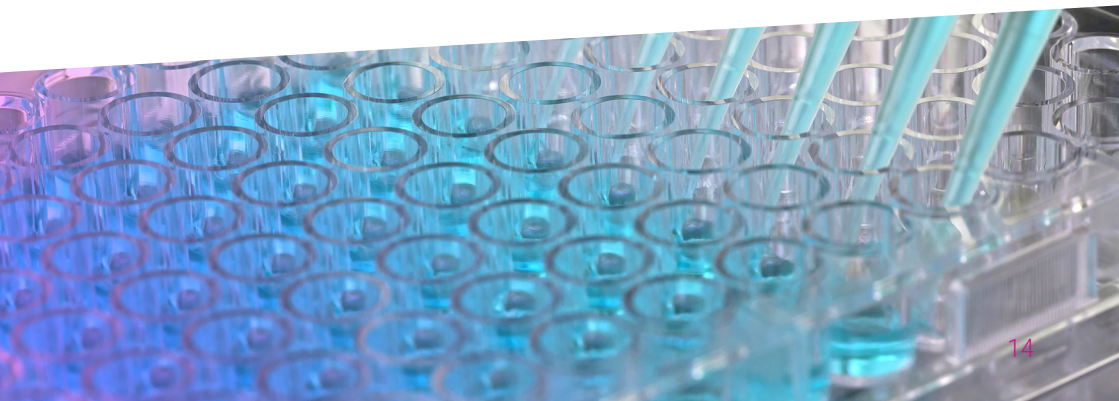
Name	Human	Mouse	Rat	Standard	Mini	ABTS	TMB
4-1BB Receptor	✓			✓		✓	
BD-1	✓			✓	✓	✓	
BD-2	✓			✓	✓	✓	✓
BD-3	✓			✓	✓	✓	
BD-4	✓			✓	✓	✓	
Betacellulin	✓			✓		✓	
BMP-2	✓	✓	✓	✓	✓	✓	✓
CNTF	✓		✓	✓	✓	✓	
CTACK (CCL27)	✓			✓	✓	✓	
CTGF	✓			✓	✓	✓	
CXCL16	✓			✓	✓	✓	
EGF	✓	✓	✓	✓	✓	✓	
EG-VEGF	✓			✓		✓	
Eotaxin (CCL11)	✓	✓		✓		✓	
Eotaxin-3 (CCL26)	✓			✓	✓	✓	
Exodus-2 (CCL21)		✓		✓	✓	✓	
FGF-basic	✓			✓	✓	✓	
Follistatin	✓			✓		✓	
G-CSF	✓	✓		✓	✓	✓	
GM-CSF	✓	✓		✓	✓	✓	
GRO/KC (CXCL1)			✓	✓		✓	
GRO-α/MGSA (CXCL1)	✓			✓		✓	
GRO-β (CXCL2)	✓			✓	✓	✓	
Heregulinβ-1	✓			✓	✓	✓	
ICAM-1	✓			✓	✓	✓	
IFN-γ	✓	✓	✓	✓	✓	✓	✓
IGF-BP1	✓			✓	✓	✓	
IGF-I		✓		✓	✓	✓	
IL-1RA	✓			✓	✓	✓	
IL-1α	✓	✓	✓	✓	✓	✓	✓
IL-1β	✓	✓	✓	✓	✓	✓	✓
IL-2	✓	✓	✓	✓	✓	✓	✓
IL-3	✓	✓		✓	✓	✓	

PeproTech ELISA Development Kits (EDK)

Name	Human	Mouse	Rat	Standard	Mini	ABTS	TMB
IL-4	✓	✓		✓	✓	✓	✓
IL-5	✓	✓		✓	✓	✓	
IL-6	✓	✓	✓	✓	✓	✓	✓
IL-7	✓			✓		✓	
IL-8 (CXCL8)	✓			✓	✓	✓	✓
IL-9	✓			✓		✓	
IL-10	✓	✓		✓	✓	✓	✓
IL-11	✓			✓	✓	✓	
IL-12	✓	✓		✓	✓	✓	✓
IL-13	✓	✓		✓	✓	✓	
IL-15		✓		✓	✓	✓	
IL-17A	✓	✓		✓	✓	✓	
IL-17E	✓			✓	✓	✓	✓
IL-17F	✓			✓		✓	
IL-20	✓			✓		✓	
IL-21	✓	✓		✓	✓	✓	
IL-22	✓	✓		✓	✓	✓	
IL-31	✓			✓	✓	✓	
IL-33	✓			✓	✓	✓	
IP-10 (CXCL10)	✓	✓	✓	✓	✓	✓	✓
I-TAC (CXCL11)	✓			✓		✓	
JE/MCP-1 (CCL2)		✓		✓	✓	✓	
KC (CXCL1)		✓		✓	✓	✓	
Leptin	✓	✓		✓		✓	
MCP-1 (CCL2)	✓		✓	✓	✓	✓	✓
MCP-2 (CCL8)	✓			✓	✓	✓	
MCP-3 (CCL7)		✓		✓		✓	
M-CSF		✓		✓		✓	
MDC (CCL22)		✓		✓		✓	
MIA-2	✓			✓		✓	
Midkine	✓			✓		✓	
MIG (CXCL9)	✓			✓	✓	✓	
MIP-1α (CCL3)	✓	✓	✓	✓	✓	✓	

PeproTech ELISA Development Kits (EDK)

Name	Human	Mouse	Rat	Standard	Mini	ABTS	TMB
MIP-1β (CCL4)		✓		✓	✓	✓	✓
MIP-2 (CXCL2)		✓		✓	✓	✓	
NAP-2 (CXCL7)	✓			✓		✓	
Neuroserpin	✓			✓	✓	✓	
NOV	✓			✓		✓	
PAI-1	✓			✓		✓	
PDGF-BB	✓			✓	✓	✓	
PlGF-1	✓			✓		✓	
RANTES (CCL5)	✓	✓	✓	✓	✓	✓	
Resistin	✓			✓	✓	✓	
sCD40 Ligand	✓			✓	✓	✓	
SCF	✓	✓	✓	✓	✓	✓	✓
SDF-1α (CXCL12)	✓			✓	✓	✓	
sRANK Ligand	✓	✓		✓	✓	✓	
sTRAIL/Apo2L	✓			✓		✓	
TACI	✓			✓		✓	
TIMP-1	✓			✓	✓	✓	
TL-1A	✓			✓	✓	✓	
TNF-α	✓	✓	✓	✓	✓	✓	✓
TPO	✓			✓	✓	✓	
TSLP	✓			✓		✓	
TWEAK	✓			✓		✓	
$VEGF_{165}$	✓	✓	✓	✓	✓	✓	✓
β-NGF	✓			✓	✓	✓	



PeproTech ELISA Components

For select proteins, that are not offered as ready made ELISA kits you can construct your own ELISA using our antigen- affinity purified antibodies, biotinylated antibodies, recombinant proteins and an HRP-conjugate (Red).

Note: Optimization might will be required. The compatibility of these components with the ELISA format may or may not have been verified by PeproTech. The other components necessary to run an ELISA can be purchased as a separate kit: ELISA Buffer Kit Catalog # 900-K00/900-T00 (see page 18).

* The ELISA Buffer Kit contains all of the necessary components to assay ten ELISA Plates

Order your ELISA components by individual catalog number:

- Recombinant protein standard
- Antigen-Affinity Purified Capture Antibody
- Biotinylated Detection Antibody
- Streptavidin/Avidin-HRP Conjugate

Additional required components

(Not supplied)

- Uncoated Microplates
- Plate Coating Buffer
- Wash Buffer
- Blocking Buffer
- Sample Diluent
- Substrate (ABTS/TMB)
- Sealing Films
- Stop solution (for TMB kits)

For ELISA Buffer Kit

* The ELISA Buffer kit contains all of the necessary components to assay ten ELISA plates. Refer to next page.*

Suggested protocol

Microplate

Capture antibody Coating buffer (PBS)	**Coating** **Overnight**
Wash buffer	**Wash**
Block buffer	**Blocking** **60 minutes**

Coated Microplate

Standard/Sample Diluent	**1st reaction** **120 minutes**
Wash buffer	**Wash**
Detection antibody Diluent	**2nd reaction** **120 minutes**
Wash buffer	**Wash**
Streptavidin/Avidin-HRP Conjugate Diluent	**3rd reaction** **30 minutes**
Wash buffer	**Wash**
Substrate (TMB/ABTS) Stop solution (only for TMB kits)	**Color reaction**
	Detection **15-60 minutes**

Available ELISA Kit Components

Name	Name	Name
Human 4-1BBL	Human GCP-2	Human MCP-4
Human Adipolean Variant	Human GDF-3	Human M-CSF
Human AITRL	Human GDNF	Human MDC
Human APO-E3	Human HCC-1	Human MEC
Human APRIL	Human Heregulin beta 1	Human MIA
Human Artemin	Human I-309	Human MIP-3
Human BAFF	Human IFN-alpha	Human MIP-3alpha
Human BCA-1	Human IFN-beta	Human MIP-3beta
Human BD-5	Human IFN-Lambda-2	Human MIP-4
Human BMP-7/OP-1	Human IGF Binding Protein 3	Human MIP-5
Human b-NGF	Human IGF Binding Protein 5	Human Nanog
Human BRAK	Human IGF-Binding Protein-1	Human Neurturin
Human Cardiotrophin-I	Human IGF-BP-7	Human NNT/BCSF-3
Human CTGFL/WISP-2	Human IGF-I	Human NP-1
Human DLL-4	Human IGF-II	Human NT-3
Human EGF Receptor	Human IL-15	Human NT-3
Human EMAPP-II	Human IL-16	Human NT-4
Human ENA-78	Human IL-17B	Human Oncostatin M
Human Endostatin	Human IL-17D	Human Osteopontin
Human Eotaxin-2	Human IL-19	Human Osteoprotegerin
Human EPO	Human IL-1Receptor Antagonist	Human p16-INK-4a TAT
Human Exodus-2	Human IL-2 sReceptor alpha	Human PDGF-AA
Human Fas Ligand	Human IL-36 gamma	Human PD-L1 Fc
Human Fas Receptor	Human KGF	Human Persephin
Human FGF-10	Human Klotho	Human PF-4
Human FGF-16	Human LD78-beta	Human PTHrP
Human FGF-4	Human LEC/NCC-4	Human Relm-beta
Human FGF-acidic	Human LIF	Human sCD14
Human flt3 Ligand	Human LIGHT	Human SCGF-beta
Human Fractalkine	Human Lymphotactin	Human SDF-1alpha
Human gAcrp30/Adipolean	Human Maspin	Human sRANK Receptor
Human Galectin-1	Human MCAF/MCP-1	Human sTNF Receptor I
Human Galectin-3	Human MCP-3	Human sTNF-Receptor II

Available ELISA Kit Components

Name	Name	Name
Human sTrail Receptor 2	Murine CTLA-4	Murine SF-20
Human TARC	Murine Eotaxin-2	Murine sTrail
Human TECK	Murine FGF-9	Rabbit Anti Human PLGF-1
Human TFF-2	Murine IL-17A	Rabbit Anti Human sCD22
Human TGF-alpha	Murine IL-18 Binding Protein	Rat GM-CSF
Human TGF-beta3	Murine IL-7	Rat GROb/MIP-2
Human TNF-beta	Murine IL-9	Rat IL-10
Human Vaspin	Murine LIX	Rat IL-13
Human VCAM-1	Murine MCP-2	Rat IL-3beta
Human Visfatin	Murine MCP-5	Rat IL-4
Human WNT-1	Murine MIP-1gamma	Rat Il-7
Human WNT-3alpha	Murine Relm-alpha	Rat Leptin
Human/Murine/Rat BDNF	Murine Relm-beta	Rat SDF-1alpha
Murine B-71	Murine Resistin	
Murine C-10	Murine SDF-1alpha	

PeproTech ELISA Buffer Kit

THE PERFECT COMPLEMENTARY KIT TO OUR EDKS AND ELISA COMPONENTS

The buffers in this supplemental kit have been specially formulated for optimal performance when used in conjunction with PeproTech's ELISA Development Kits. The buffer kit contains all of the necessary components to assay ten 96-well ELISA plates (included) and contains detailed handling instructions. All of the reagents have been sterile-filtered to minimize assay interference and maximize shelf life.

This easy-to-use ELISA buffer kit can also be purchased as a stand-alone product since the plates included are not pre-coated with capture antibody. The flexibility of this format allows the researcher to develop and optimize an ELISA with their own capture and detection antibodies. The antibody concentrations needed and the detection ranges of the ELISA will vary according to the specific components used.

Kit contents:

- 20X Plate Coating Buffer
- 1X Blocking Buffer
- 20X Sample Diluent
- 20X Wash Buffer
- Ready-to-use ABTS or TMB
- 96 well ELISA Plates
- 50 Plate Sealing Films
- Buffer Handling Instructions
- Stop solution (for TMB kits)

TMB ELISA Buffer Kit	Cat. # 900-T00	Price: $195
ABTS ELISA Buffer Kit	Cat. # 900-K00	Price: $195

PeproTech ELISA Tips

Always wear gloves and use clean labwear and pipet tips in order to protect solutions from contamination with proteins commonly found on the skin, particularly proteases and chemicals from the work environment.

Use flat-bottomed, 96-well plates, made from polystyrene. Avoid using tissue - culture treated plates. It is best to use plates intended specifically for ELISA since they are designed to have high protein-binding capacity, batch-to-batch consistency and optimal optical conditions.

The "edge effect" occurs when uneven assay conditions cause the outer wells to behave differently from the inner wells. This can result in unexpected values in the outer wells, which are out of line with neighboring wells. The most probable causes of this effect are temperature differences or uneven evaporation between the peripheral and the central wells. Temperature differences are caused due to polystyrene's poor heat conductance, especially when the liquid reagents are used straight from a refrigerator and then incubated at 37°C or when plates are stacked. The difference in evaporation is due to increased evaporation rate of peripheral wells compared to the centrally-located wells. In order to avoid edge effect the liquid reagents (and plates) should be brought to the temperature intended for incubation and the plates should be sealed with adhesive tape or placed in a 100% relative humidity environment during incubation.

The high dose "hook effect" refers to a "hook" that is observed in the curve that plots signal versus antigen concentration, when the higher levels of antigen display a significantly lower signal than the actual level present in the sample. This phenomenon typically happens in a one- step ELISA when the sample and the detection antibody are added simultaneously. The excess antigen binds to all available sites on both the capture antibody as well as the detection antibody and thereby prevents the sandwich-formation and results in a falsely low signal. In a two-step sandwich ELISA this effect might result from reduced binding due to steric interference of the excess antigen. When there is a possibility of a very high concentration of the target antigen in the sample it is recommended to test several dilutions of the sample in order to avoid this issue.

One of the most common sources of assay imprecision is pipetting error. Whenever possible use a multichannel pipettor in order to reduce the number of pipetting steps. It is essential to ensure that the pipette tips are properly fastened since a loose tip leads to inaccurate volume pick-up and delivery. This is particularly important when using multichannel pipettors as often the tips in the end rows do not attach fully to the pipettor. It is a good practice to observe the level of the liquid in the pipette tips and the wells while applying the liquid reagents in order to make sure that all the wells receive the same volume of liquid reagent or sample.

Carryover is the process by which materials, such as parts of a sample or reaction reagent, are unintentionally

PeproTech ELISA Tips - Continued

transferred from one well into another and produce erroneous results. In order to eliminate the possibility of carryover, pipette tips should be changed between samples and splashing during pipetting or vigorous shaking of the plate that might result in transfer of liquid to adjacent wells should be avoided.

A complex heterogeneous matrix, such as a crude antibody preparation or whole serum is less suitable for coating since the effective antigen/antibody concentration may be outcompeted by other proteins. This will result in a low amount of immobilization, which will render the specific assay signal too low to be useful.

When possible, it is advisable to prepare the standard curve in the same sample matrix in order to minimize matrix effects and obtain more accurate quantification. For example, when detecting cytokine secretion, the standard should be diluted in fresh cell culture medium.

Blocking is necessary to prevent non-specific binding of the detection antibodies to the well surface. Blocking buffers should contain unrelated protein or protein derivatives that are not expected to react with any of the antibodies being used in the detection step. The most commonly used protein blockers are bovine serum albumin (BSA), non-fat dry milk or casein, whole normal serum, and fish gelatin. Each of these blockers has its own advantages and disadvantages, which should be taken into consideration when developing an ELISA.

One of the key steps for a successful ELISA is washing. The most commonly used wash buffer is PBS with a small concentration of Tween-20. The washing steps are essential for reducing background signal and thereby increase the assay's signal-to-noise ratio. Washing between incubation steps ensures that only the specific binding events are maintained, providing a signal at the final step which is proportional to the concentration of the target analyte in the sample. The wash volume should be at least as high, or preferably higher, than the coating volume of the well. A volume too small leaves part of the coated well surface unwashed and significantly increases the background. Insufficient washing often results in increased variation and high background, which leads to poor results. On the other hand, too extensive washing might reduce signal strength. If specific wash instructions are not available, as a good rule of thumb, start with three wash cycles for every wash step and increase the number of cycles if the background is too high. Excess wash solution must be removed in the final wash step to prevent the dilution of the reagents added in the subsequent step.
This is usually accomplished by tapping the washed plate upside down on an absorbent paper to remove excess liquid.

Read the plates as soon as possible following the addition of stop solution. Before reading, carefully wipe the bottom of the plate to ensure no interference of the reading by moisture or fingerprints.

PeproTech ELISA Tips - Continued

Avoid touching the side walls and the bottom of the well with tips when applying reagents since scratching the surface might remove binding from previous steps leading to false results and increased variation.

Use standard (forward) pipetting for the preparation of sample dilutions and reverse pipetting for the addition of samples and reagents (conjugate, substrate and stop) in order to avoid bubbles in the wells. Reverse pipetting is performed by:

1. Press the operating button to the second stop.
2. Dip the tip into the reagent solution and slowly release the operating button to fill the tip. Withdraw the tip from the liquid, making sure to remove excess liquid outside the tip.
3. Dispense the liquid into the receiving well by gently pushing the operating button to the first stop. Hold the button in this position and don't dispense the solution remaining in the tip.
4. For repetitive pipetting repeat steps 2 and 3 while inspecting the liquid level in the tips in order to ensure that all the wells receive the same volume of liquid.
5. When the dispensing is completed discard the tip with the excess liquid to the waste.

Minimize the risk of contamination of by labeling the reagent reservoirs and changing tips for each reagent.

When an assay is routinely run over a period of time it is recommended to apply positive and negative controls in order to monitor the ELISA performance and to be able to compare results run on different plates and dates.

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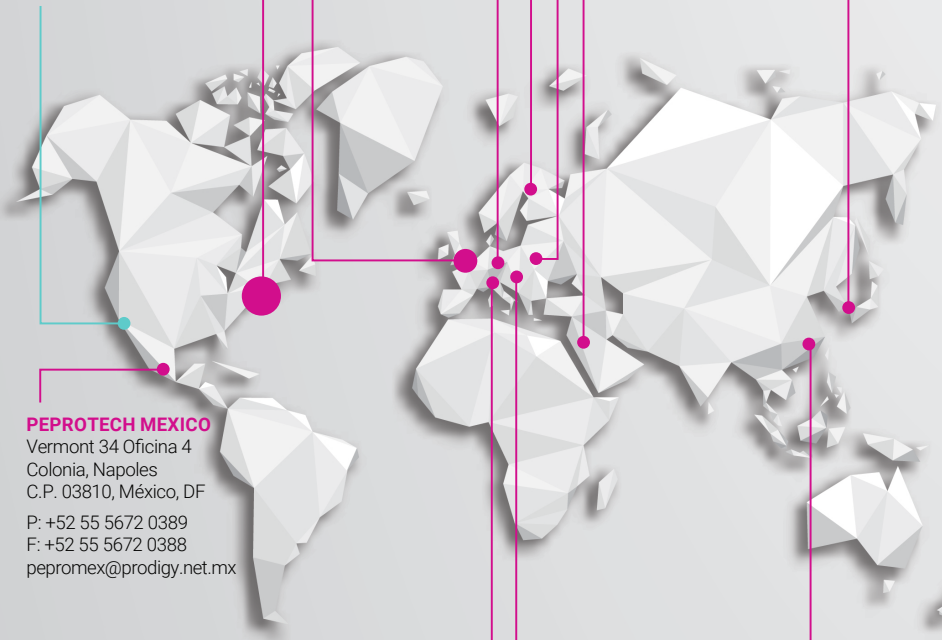

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