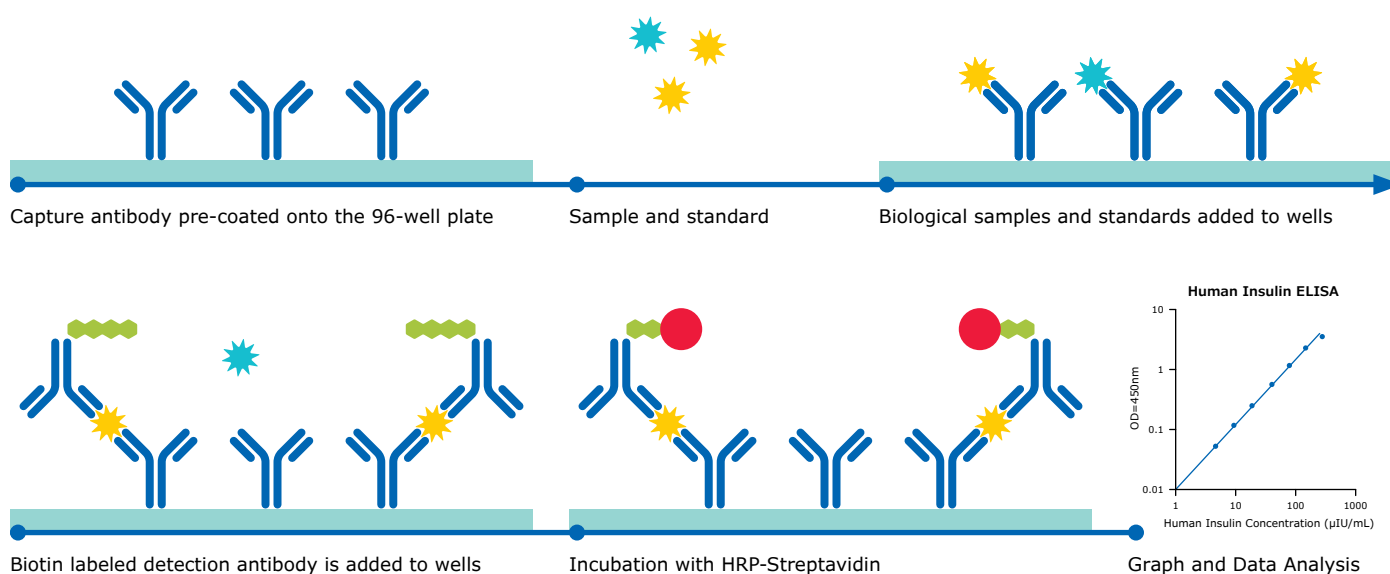# ELISAs for Research Use

Millipore®

Preparation, Separation, Filtration & Testing Products

An ELISA (Enzyme-Linked Immunosorbent Assay) is a plate-based immunoassay within which one of the assay components, typically an antibody, is adsorbed onto a solid surface, in this case, a plate. Offering rapid, quantitative and sensitive analyte detection at relatively low cost, ELISAs represent one of the simplest assay formats to perform. Furthermore, the ease of adapting an ELISA to a higher throughput screening method empowers researchers to test large sample numbers in a single run.
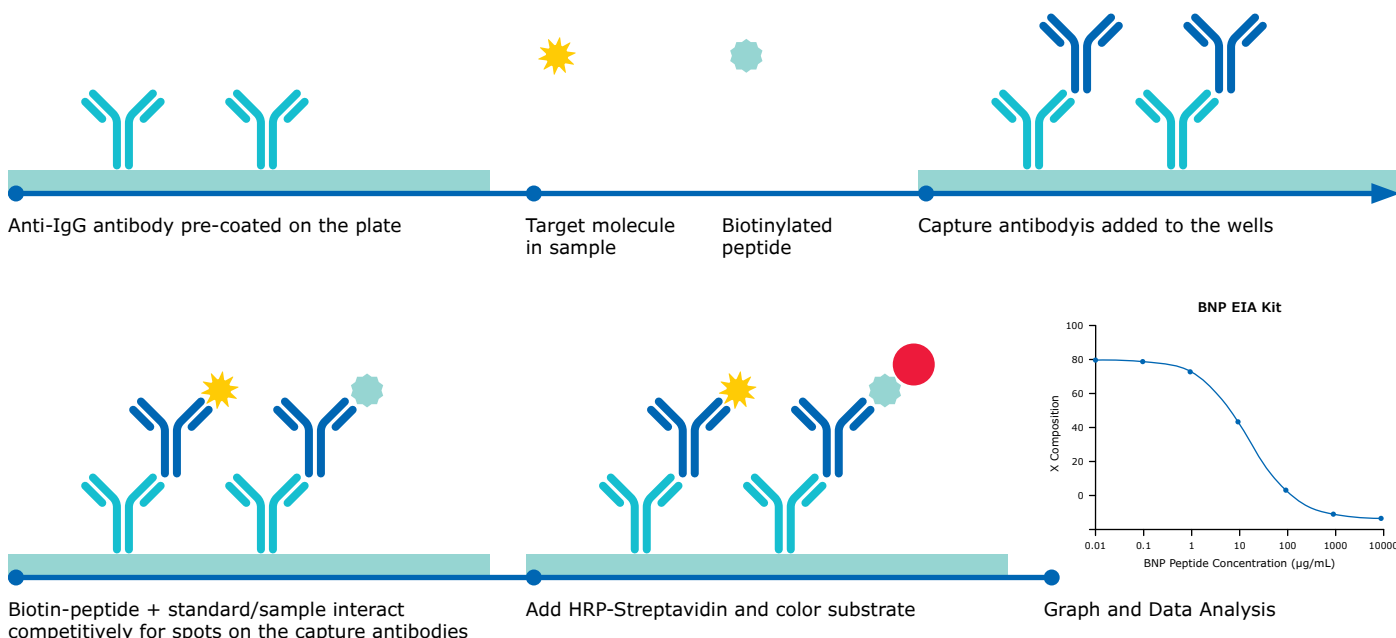
ELISA formats

There are several ELISA configurations, with the most common formats being sandwich, competitive and signaling assays. Concentration-dependent detection of the target analyte is accompanied by either an increase or a decrease in signal, which is related to the format that is chosen.

Sandwich ELISA

Capture antibody pre-coated onto the 96-well plate   Sample and standard   Biological samples and standards added to wells

Biotin labeled detection antibody is added to wells   Incubation with HRP-Streptavidin   Graph and Data Analysis

Human Insulin ELISA
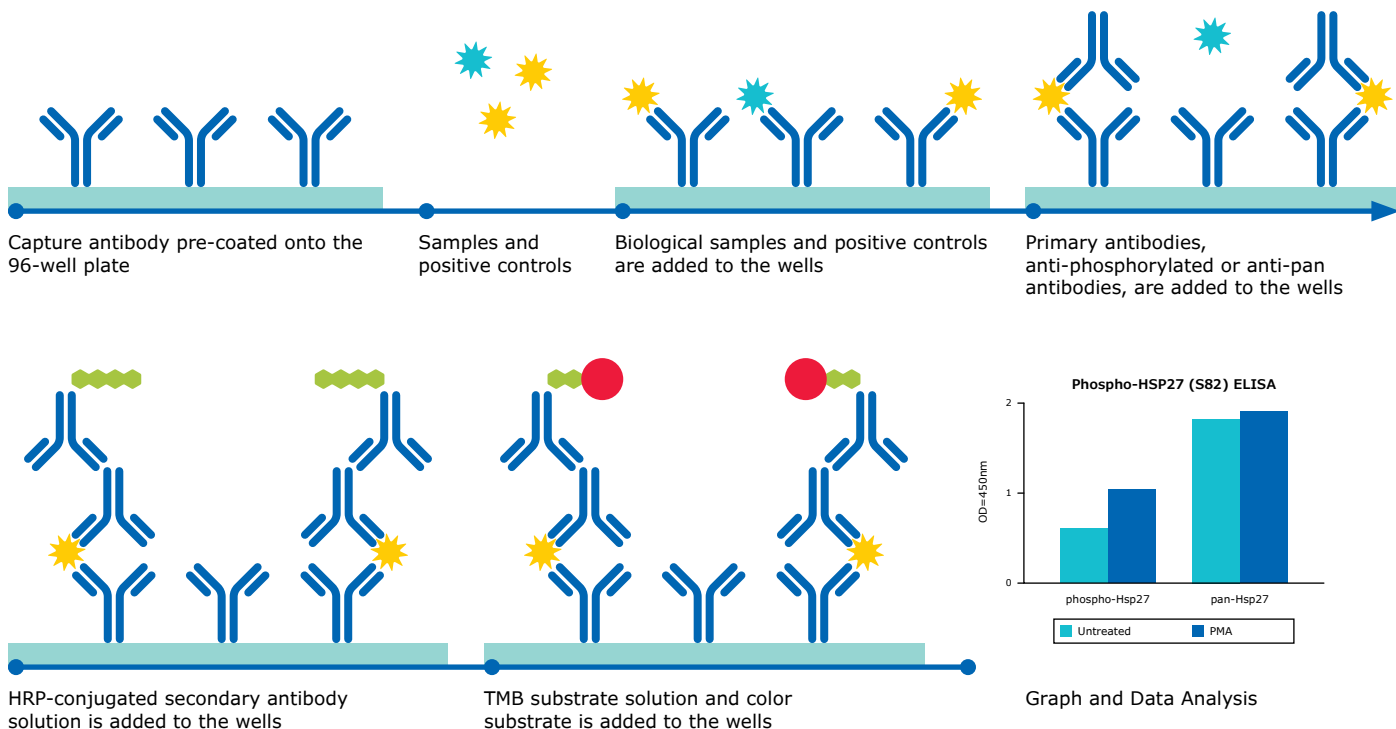
OD=450nm / Human Insulin Concentration (µIU/mL)

- Uses two analyte-specific antibodies, each recognizing a different epitope of the target biomarker
- As the analyte concentration within the sample increases, an increase in signal is observed

Competitive ELISA

Anti-IgG antibody pre-coated on the plate

Target molecule in sample

Biotinylated peptide

Capture antibodyis added to the wells

Biotin-peptide + standard/sample interact competitively for spots on the capture antibodies

Add HRP-Streptavidin and color substrate

BNP EIA Kit

X Composition

BNP Peptide Concentration (µg/mL)

Graph and Data Analysis

- Analyte that is present in the sample competes with a known amount of labeled reference analyte for antibody binding
- As the analyte concentration within the sample increases, a decrease in signal is observed

Signaling ELISA

Capture antibody pre-coated onto the 96-well plate

Samples and positive controls

Biological samples and positive controls are added to the wells

Primary antibodies, anti-phosphorylated or anti-pan antibodies, are added to the wells

HRP-conjugated secondary antibody solution is added to the wells

TMB substrate solution and color substrate is added to the wells

Phospho-HSP27 (S82) ELISA

OD=450nm

phospho-Hsp27   pan-Hsp27

Untreated   PMA

Graph and Data Analysis

- Often used to study phosphorylation-based signaling, two analyte-specific antibodies are used to detect total (pan) and phosphorylated analyte following capture on the microplate (note that this detection is not simultaneous)
- Levels of phosphorylated analyte can easily be normalized to total analyte levels

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Our ELISA portfolio

Covering a diversity of research areas and species, our pre-validated, easy to use ELISAs deliver reliable and reproducible results for quantifying biomarkers in complex sample types at the pg/mL level. Our substantial portfolio currently includes over 1,800 sensitive and specific ELISAs - the easiest way to search them is at **SigmaAldrich.com/ELISA**.
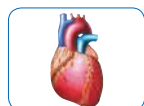
Research areas covered
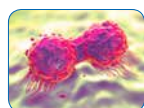
Cancer

Toxicity

Neuroscience

Bone Metabolism

Cardiovascular Disease

Immunology

- Acute Phase Proteins
- Adipokines
- Angiogenesis Biomarkers
- Apolipoproteins
- Apoptosis Pathway Proteins
- BMPs
- Bone Metabolism Biomarkers
- Cancer Biomarkers
- Cardiovascular Disease Biomarkers
- CD Markers
- Cell Signaling Pathway Proteins
- Cell Structure Proteins
- Chemokines
- Complement Factors
- Cytokines

- Growth Factors
- Gut Hormones
- IGFBPs
- Immune Checkpoint Proteins
- Immunoglobulins
- Kidney Injury Biomarkers
- Liver Proteins
- Metabolic Hormones
- MMPs
- Neurodegenerative Disease Biomarkers
- Neurological Disorders Biomarkers
- Sepsis Biomarkers
- Soluble Receptors
- Thyroid Hormones
- Vascular Injury Biomarkers

Species within our portfolio

	Cytokines	Metabolic	Cardiovascular	Angiogenesis	Signaling
	✔	✔	✔	✔	✔
	✔	✔	✔	✔	✔
	✔	✔		✔	✔
	✔				
	✔	✔			
	✔				
	✔				
	✔				
	✔				
	✔	✔			

Consistency and reliability

While all the ELISAs within our extensive portfolio are backed by award-winning technical resources, a proportion of these kits consist of ELISAs from Merck Millipore. Developed and manufactured in the USA, these products have roots stretching back to Linco Research, Inc (a spin-off from Washington University in St. Louis in 1978) and Upstate Biotechnology™ (founded in 1996 and subsequently acquired by Merck Millipore in 2006) and are manufactured according to strict validation criteria.

Our rigorous validation criteria

✔ Inter- and intra-assay CV

✔ Biological range

✔ Sample stability

✔ Sensitivity

✔ Cross-reactivity

✔ Shipping stability

✔ Recovery & Linearity

✔ Species-specificity

✔ Method comparison

Analytes covered by our Merck Millipore® ELISAs

Metabolic

Adiponectin	GIP (Total)	Leptin
Adiponectin HMW	GLP-1 (active)	Pancreatic Polypeptide
Amylin (Active)	GLP-1 HS (Active)	Procollagen Type IIA N-Propeptide
C-Peptide	GLP-1 (Total)	Proinsulin (Total)
C-Peptide 2	GLP-2	PYY (Total)
FGF-21	Glucagon	Resistin
FGF-23	Growth Hormone	SAA-3
Ghrelin (Active)	Insulin	
Ghrelin (Total)	Insulin (Serum Free)	

Neuroscience

α-Synuclein	GFAP	pNF-H
Amyloid beta 1-40	NGF	S100B
Amyloid beta 1-42	NPY	
BDNF	PEDF	

Species available are human, mouse and rat for our metabolic kits, with the inclusion of canine for our neuroscience kits (note that not all analytes are available in all species)

Our strict R&D validation metrics and meticulous manufacturing practices ensure that our legacy ELISAs demonstrate consistent lot-to-lot performance within an appropriate biological range, to defined feasibility criteria over the lifetime of the product. This is illustrated by our High Molecular Weight Adiponectin kit.

## Consistent lot-to-lot performance within an appropriate biological range

**Human HMW Adiponectin ELISA
E8065-K Standard First Lot**
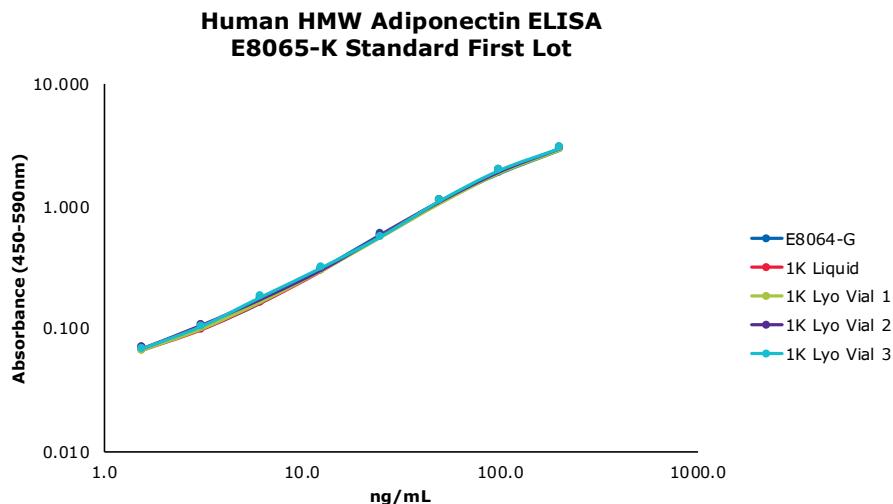
Preparations of multiple calibrator lots from liquid to lyophilised (finished) versions for use in EZHMWAN-65K, monitored against a gold standard.

Defined feasibility criteria guarantee reliable and reproducible data

	QC1	QC2
Inter-assay CV	7.40%	10.60%
Intra-assay CV	2.50%	3.40%
Sensitivity	0.7ng	1.5ng (+2SD)
Recovery linearity	104%	106%

Enhanced validation through endogenous sample testing on relevant patient samples

**Human HMW Adiponectin ELISA
Pre-treated Samples (1:200)**
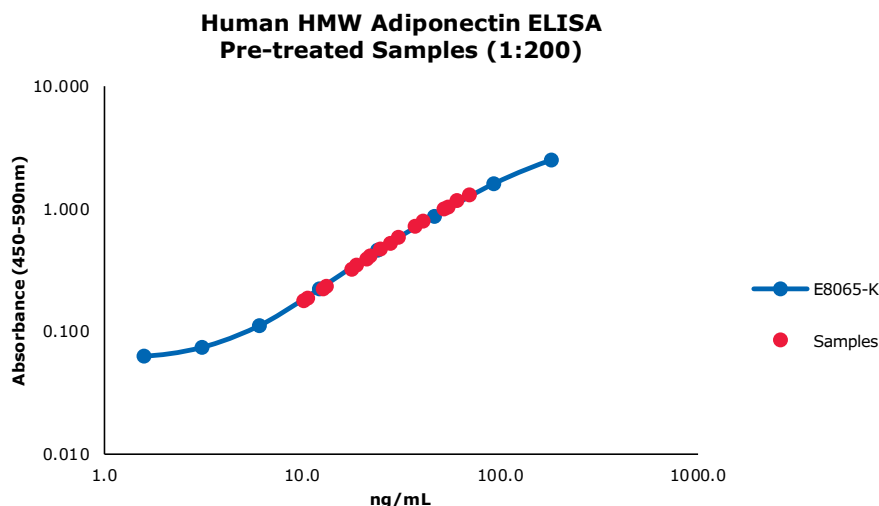
Performance of pre- and post-fasting human serum and plasma samples (n=8) against a standard curve of EZHMWAN-65K

Supporting your ELISA research at every step

Accurate reagent dispensing and thorough plate washing are essential to the success of any ELISA. To avoid the variability that can be introduced when performing these processes manually, we offer a choice of reagent dispensing and washing solutions through our partnership with BioTek. A well-respected company established in 1968, all BioTek's research and manufacturing is carried out in Vermont, USA.

Included within this range of products is BioTek's popular and user-friendly 50™ TS plate washer. We also offer BioTek's latest benchtop absorbance reader, the 800™ TS, which is compatible with the majority of our ELISAs.

50™ TS Plate Washer

BioTek's 50™ TS plate washer is a compact microplate washing system which facilitates the fast and intuitive creation of user-defined protocols. Utilizing a color touchscreen, with menu-driven programming, it represents an affordable choice for exceptional ELISA performance and brings convenience and consistently high-quality results to plate washing workflows.

Cat. No.	Description
40-301	BioTek® 50 TS Plate Washer*

- Color touchscreen for quick and easy programming and simple operation
- Easy touch operation for washing full or partial plates
- Liquid level sensing for reliable and safe performance
- Automated switching of up to 3 buffers for even greater automation
- Automated, built-in maintenance routines for continued reliable operation
- Application versatility: ELISA, cell-based assays and bead-based assays

800™ TS Absorbance Reader

BioTek's 800™ TS benchtop reader is supplied with a preset configuration of four filters (450, 590, 630 & 750nm), allowing the absorbance of the majority of our ELISAs to be read. The platform is available as a variety of options, ranging from the basic reader to the higher end CFR 21 Part 11 compliant model with Gen 5 BioTek software and a NIST traceable absorbance test plate for those in a more regulated environment. The Gen 5 software represents BioTek's latest acquisition package, improving the resolution of absorbance from 0.01 to 0.001 OD.

Cat. No.	Description
40-300	BioTek® 800 TS Microplate Reader, Basic Version
40-006	BioTek® 800 TS Microplate Reader with Gen 5 Software
40-007	BioTek® 800 TS Microplate Reader with Gen 5 Secure and Validation Package

- Color touchscreen for quick and easy programming and simple operation
- Ideal for ELISA and applications including end-point assays, kinetics and cell-based assays
- Available as a variety of options, including NIST traceable absorbance test plate
- High precision and accuracy for reliable results
- USB flash drive for convenient data export, Gen5 import for analysis
- Durable and high-quality design

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