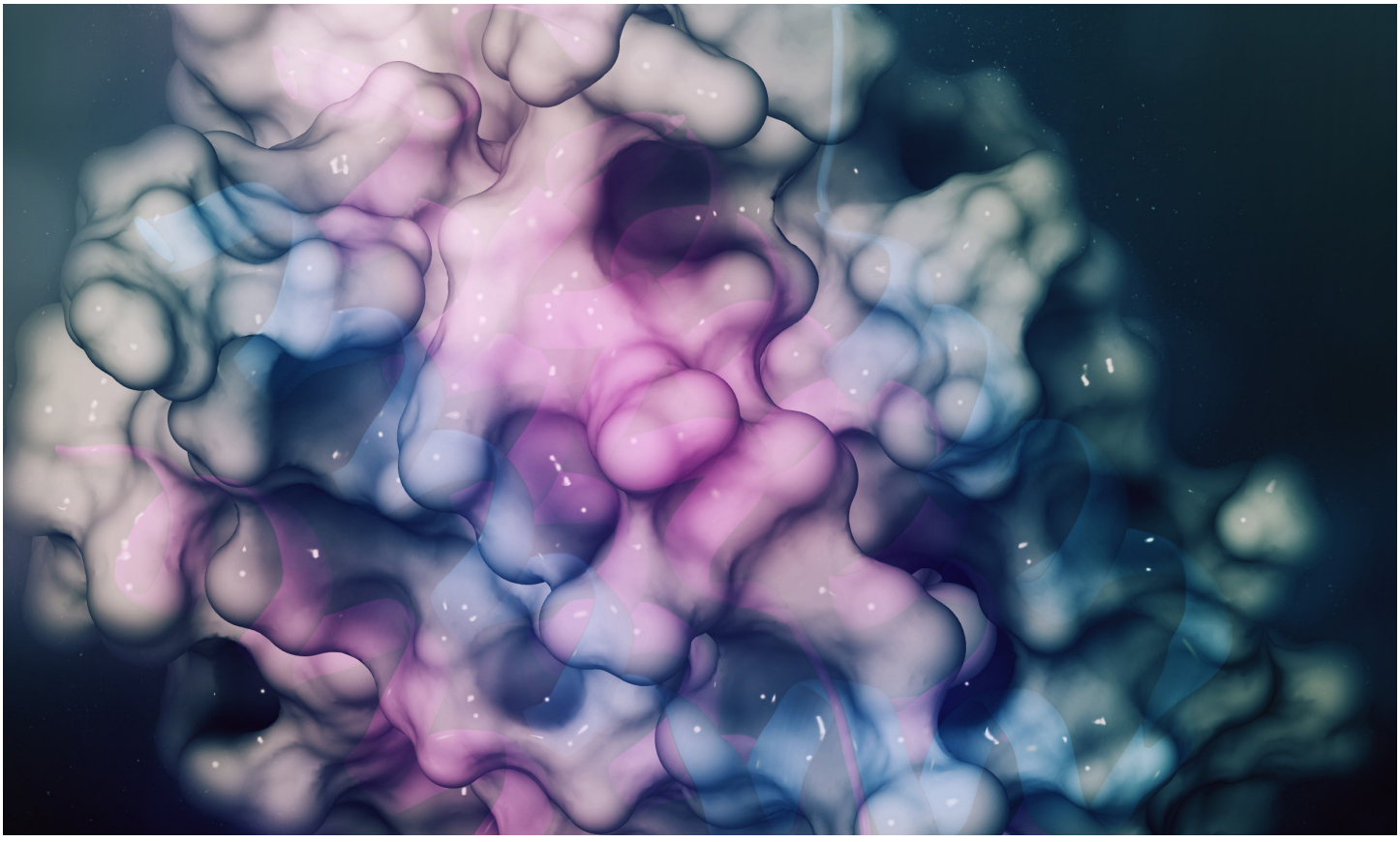
Proteomics

Custom peptide synthesis

Standard | modified | heavy peptides | libraries

thermo scientific

Contents



Custom peptide synthesis

An overview

Optimizing the efficiency of peptide synthesis can be challenging. At Thermo Fisher Scientific, we have the experience, equipment, and knowledge to meet your needs for custom peptide synthesis. Our synthesis team has accumulated significant expertise through successfully producing tens of thousands of custom peptides. We are constantly adapting our product offerings to your needs based on your input. Our experienced peptide scientists will support you with peptide sequence, scale, and/or purity selections for your assay to help you achieve the best results for your application (Table 1).

Custom peptide synthesis capabilities

Table 1. Summary of peptide services and specifications.

	Standard Peptides	PEPotec Immuno Peptide Libraries	PEPotec SRM Peptide Libraries	HeavyPeptide and LightPeptide AQUA Standards
Primary application	Basic research or biomarker discovery	Epitope mapping, vaccine development	Discovery	Quantitative mass spectrometry
Key features	Over 400 modifications available	High throughput, low toxicity	High throughput	High quality and concentration precision
Peptide length	2–110	6–25	6–25	Up to 15*
Manufacturing scale	1 mg–1 kg+**	1 mg	≥0.1 mg	Multiple scales available**
Purity	Crude to >98%	Crude (as synthesized)	Crude (as synthesized)	>95% to >97%
Quality control	MS and HPLC	MS	MS check or analysis	MS, HPLC, AAA
Production time	2–3 weeks	4 weeks	2–3 weeks	2–3 weeks AQUA Express*** 5–7 weeks standard AQUA

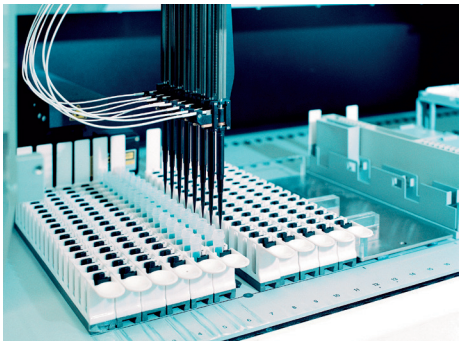
* Please inquire about longer peptide lengths.
** Greater amounts available upon request.
*** AQUA Express requests are available for up to 10 sequences on a single order and must be approved for express turnaround time.

Quality control

Quality control for our peptide services includes analyses using mass spectrometry (MS) for peptide identification, analytical high-performance liquid chromatography (HPLC) for peptide purity, and amino acid analysis (AAA) for peptide concentration. Additional nonstandard analyses are available on request. Analysis results are reported on the Certificate of Analysis for your records.

Delivery format

Our peptides are provided in lyophilized and liquid formats in glass vials as well as in 96-well Thermo Scientific™ Matrix™ tube plates, depending on the product category. Please inquire for additional formats and other custom requests.



Custom peptide synthesis, cont.

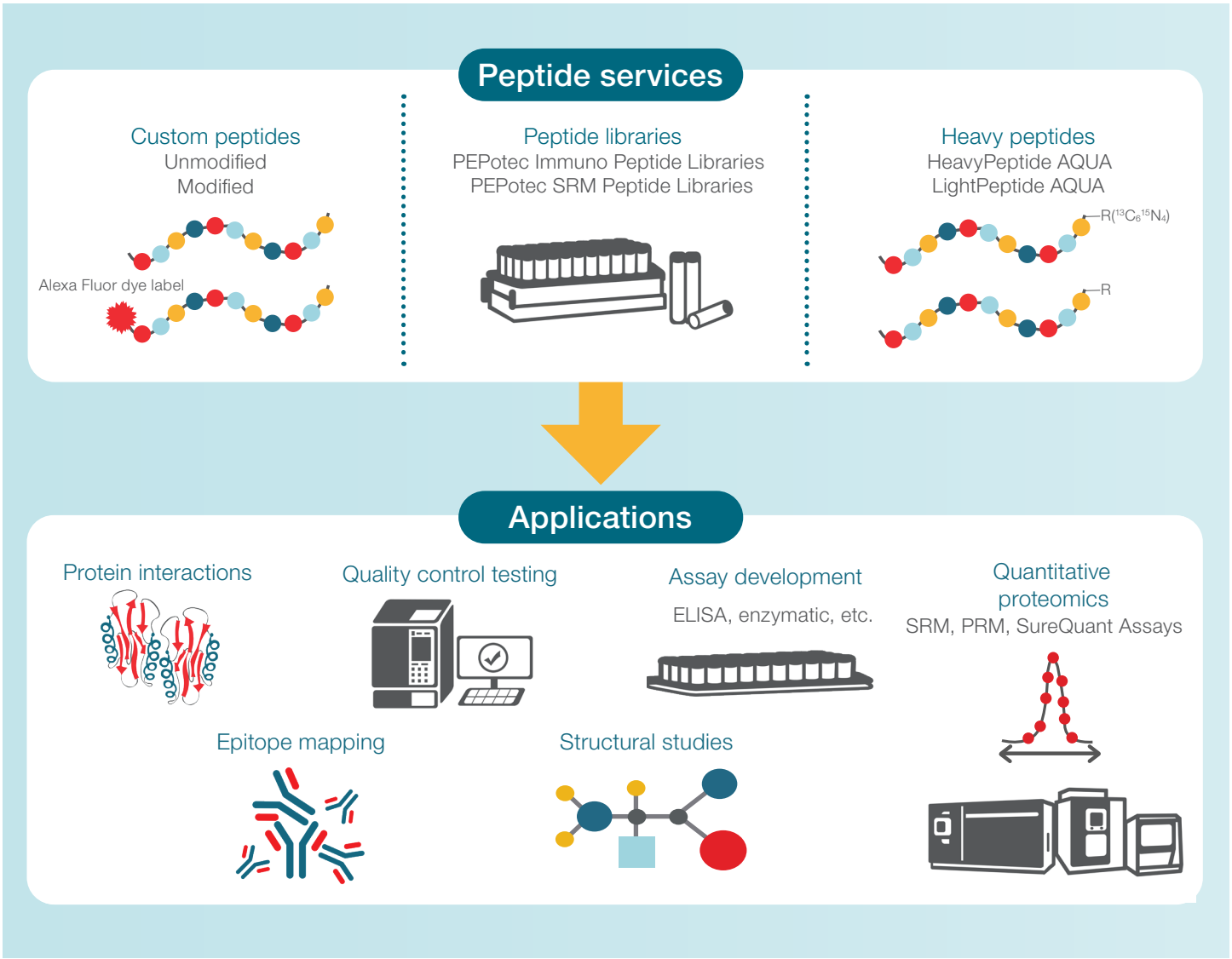
An overview

Professional support

We have experienced and dedicated staff to assist you through peptide selection, ordering, tracking, and delivery processes. We realize that the process can be lengthy, and we strive to keep you informed every step of the way.

Peptide applications

The use of synthetic peptides has significantly impacted many areas of research. Our portfolio of custom peptide services supports a variety of end applications, and we strive to provide high-quality, fit-for-purpose peptides to enable success.



Standard peptide synthesis service

High-quality peptides from validated process tailored to help meet your needs

The Thermo Scientific™ Standard Peptide Synthesis Service offers numerous options for peptide purity, modifications, and formats, providing you the flexibility to meet your research needs.

Our peptide synthesis utilizes the latest Fmoc solid-phase technology and reversed-phase HPLC for peptide purification. Peptide sequence is confirmed by MS, and peptide purity is determined using analytical HPLC.

Highlights

- **High quality**—all peptides are synthesized with high-quality materials
- **Modifications**—most comprehensive list of available modifications and labels
- **Validated**—all peptides are analyzed by MS alone or in combination with analytical HPLC
- **Flexible**—peptides available in a variety of formats and purities; manual synthesis available for difficult peptides

Modifications

We offer a wide range of N-terminal, C-terminal, and other modifications with our standard peptide service (Table 3). Please go to **thermofisher.com/peptides** for the most up-to-date list and available positions in the peptide sequence.

Custom conjugation services are also available, including conjugation to carrier proteins (KLH, BSA, OVA, or Thermo Scientific™ Imject™ Blue Carrier™ Protein) and MAPs.

Table 2. Specifications for standard grade peptides.

Peptide length	2–110 amino acids, L- or D-isoforms
Manufacturing scale*	1 mg–1 kg+
Purity	Crude to >98% pure
Counterion	TFA
Formulation	Lyophilized
Delivery format	Glass vial
Production time	2–3 weeks
Shipment	Lyophilized at room temperature
Quality control	MS for crude peptides; MS and HPLC for all other grades of purity
Modifications	Extensive list of modifications and labels, including heavy peptides (isotope-labeled)
Conjugation	Custom conjugation available, e.g., protein-peptide conjugates and multiple antigen peptides (MAPs)
Optional services	Alternate counterion, AAA, endotoxin testing, solubility testing

* Greater amounts available upon request.

Standard peptide synthesis service, cont.

Table 3. Peptide modifications offered with standard peptide synthesis.

- Acetylation of N terminus (Ac-NH–)
- Acetyl-lysine
- Aldehyde
- Invitrogen™ Alexa Fluor™ dyes
- 6-amino hexanoic acid (Ahx)
- 6-amino caproic acid (Aca)
- Amidation of C terminus (–$CONH_2$)
- Amino benzoic acid
- Beta-alanine
- Biotin
- Carbamido methylation (CAM)
- Citrulline
- Conjugation to proteins (BSA, KLH)
- Conjugation to oligonucleotides
- Custom FRET peptides
- Cyclization via termini or disulfide bridge
- D amino acids
- Dabcyl
- Dabsyl
- Dansyl
- Dihydroxy tyrosine
- Dimethyl lysine
- Dinitrophenyl (DNP)
- EDANS
- Farnesyl
- Fluorescein (FITC/5-FAM)
- Hydrocarbon spacers
- Hydroxy proline
- Hydroxy tryptophan
- Isotopically labeled amino acids (with ^{13}C, ^{15}N)
- Mercaptopropionic acid
- Methoxy-coumarin-acetic acid (MCA)
- Methionine sulfone
- Methionine sulfoxide [Met(O)]
- Monomethyl lysine
- Monomethyl arginine
- Multiple antigen peptides (MAPs)
- Norleucine (Nle)
- Phosphorylation of serine, threonine, and tyrosine
- Polyethylene glycol (PEG) spacer
- Pyroglutamic acid (Pyr)
- Rhodamine
- Special amino acids (D amino acids, other amino acids)
- Tetramethylrhodamine (TAMRA)
- Invitrogen™ Texas Red™ dye
- Ubiquitination
- Other dyes or modifications on request

Table 4. Peptide purity recommendations by application.

Crude: peptides for high-throughput screening
>70%: antigens for polyclonal antibody production
>75%: ligands for affinity purification, enzyme substrate studies, epitope mapping
>80%: qualitative peptide blocking studies, protein electrophoresis applications
>85%: immunological applications, peptide arrays, affinity purification
>90%: qualitative bioassays, monoclonal antibody production
>95%: quantitative bioassays (e.g., ELISA, enzymology, biological activity, etc.)
>97%: MS proteomics applications
>98%: structural studies, crystallography, NMR, *in vivo* applications

Note: Listed purity levels indicate the minimum typical requirements for each application.

Learn more at **thermofisher.com/standardpeptide**

PEPotec Immuno Peptide Libraries

Ideal for epitope mapping and high-throughput screening for immunology applications

Thermo Scientific™ PEPotec™ Immuno Peptide Libraries are fully synthetic custom libraries that support high-throughput screening assays to map epitopes or identify immunogenic sites in proteins. These libraries are fully customizable and supplied with acetate as the counterion to avoid potential toxicity issues in cell-based applications.

Highlights

- **Low toxicity**—acetate used as the counterion to reduce toxicity when compared to trifluoroacetate (TFA) [1]
- **Convenient**—lyophilized peptides provided in individual 2D barcoded tubes in 96-tube plates
- **Flexible**—optional services, including phosphorylation and acetylation, are available for studying signaling and regulatory proteins

Applications

- Epitope mapping of B and T cell
- Vaccine development
- High-throughput peptide screening
- Biomarker discovery
- Cell signaling (kinase/protease studies)

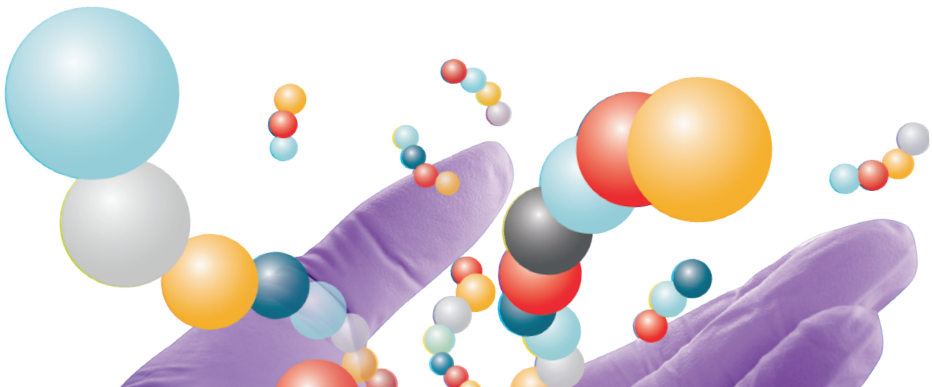
Table 5. PEPotec Immuno Peptide Library service.

Peptide length	6–25 amino acids; L-isoforms only
Quantity	1 mg
Purity	Crude (as synthesized)
C-terminal residue	Any unmodified L-isoform amino acid
Counterion	Acetate
Formulation	Lyophilized
Delivery format	Peptides are provided in individual 2-D barcoded tubes in Thermo Scientific Matrix 96-tube plates
Production time	4 weeks
Shipment	Lyophilized at room temperature
Minimum order	24 peptides; surcharge for orders of <48 peptides
Quality control	MS
Modifications	Acetylated Lys, carbamidomethylated Cys, phorphorylation, etc.

Reference

1. Pini A et al. (2012) Efficacy and toxicity of the antimicrobial peptide M33 produced with different counter-ions. *Amino Acids* 43:467–473.

Learn more at **thermofisher.com/pepotec-immuno**



Targeted quantitation

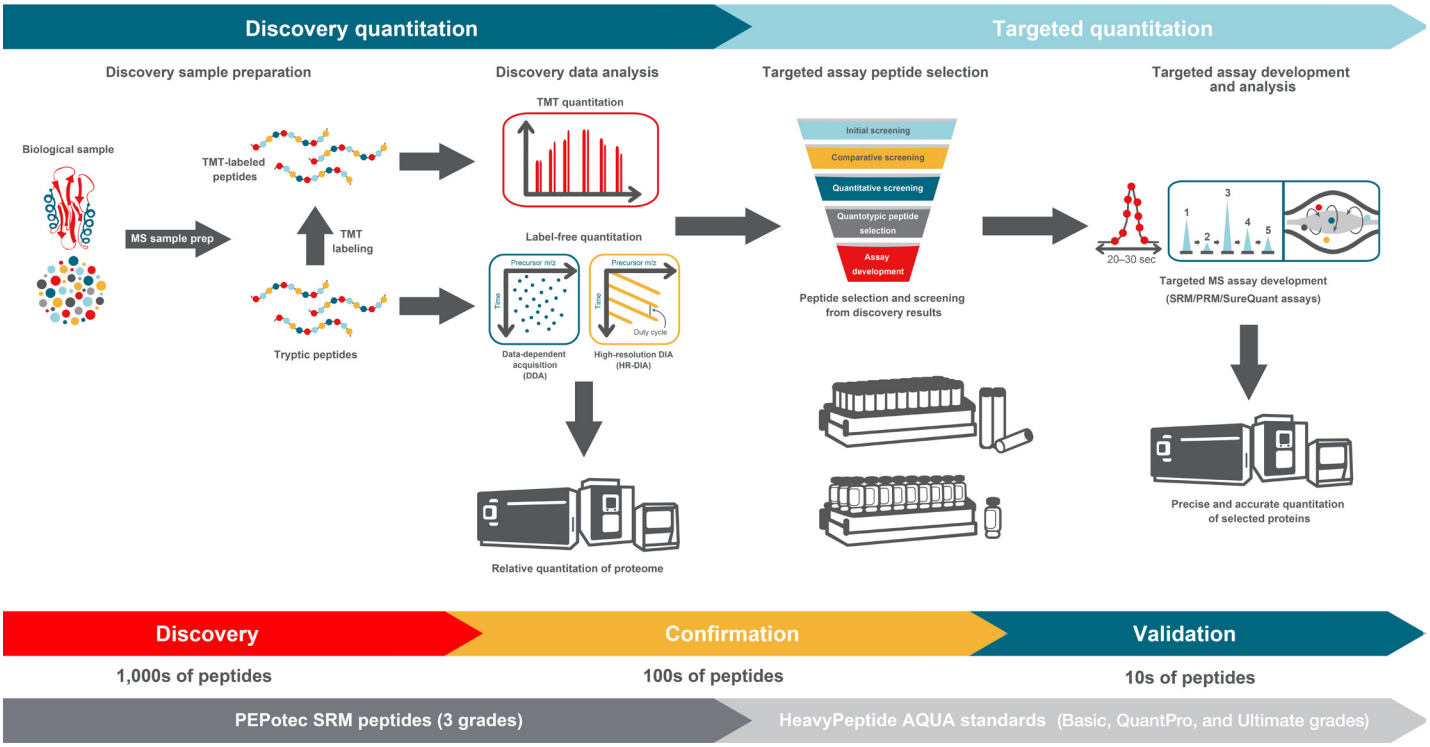
Quantitation of selected proteins

While discovery proteomics globally provides relative quantitation for entire proteomes, targeted proteomics focuses on the quantitation of specific proteins with high precision, sensitivity, and selectivity.

The goal of targeted quantitative analyses is to investigate selected protein targets from discovery or hypothesis-driven studies, to understand biological pathways or to verify and select biomarkers from multiple biological sources and conditions.

Synthetic peptides are an integral part of targeted assay design and development. The traditional targeted assay experimental design begins with selection of proteotypic peptides through software-assisted selection or utilization of data from discovery proteomics experiments. These selected peptides are then often inexpensively screened using a crude peptide library. After optimal peptide sequence selection, highly pure heavy peptides of the best candidates are synthesized and purified for target quantitation. The heavy peptides serve as internal quantitative standards for absolute quantification of the corresponding natural peptides in a biological sample. We offer products that enable assay development from validation to quantitation, including Thermo Scientific™ SRM Peptide Libraries and Thermo Scientific™ HeavyPeptide™ and LightPeptide™ AQUA Standards for quantitation.

Targeted quantitative analyses require fast, robust, and cost-effective platforms, and both HRAM Orbitrap mass spectrometers and triple quadrupole mass spectrometers are ideally suited for quantitative analysis. Triple quadrupole mass spectrometers identify and quantitate peptides by serially monitoring specific mass windows for peptides of interest, isolating and fragmenting, followed by quantitation. This selective reaction monitoring (SRM) strategy for targeted quantitation, along with chromatographic retention time information, provides high sensitivity and specificity. HRAM Orbitrap instrumentation quantifies proteins using parallel reaction monitoring (PRM). A PRM assay is different from an SRM assay in that all fragmented ions from a selected precursor ion are monitored, rather than a selected fragmented ion from a selected precursor ion. As a result, the ability to analyze all ions via PRM results in higher specificity and faster development time, but with a trade-off in sensitivity.



HeavyPeptide and LightPeptide AQUA standards

Improve quantitative precision for absolute quantitation

The HeavyPeptide and LightPeptide AQUA custom synthesis services provide isotopically labeled or unlabeled AQUA (Absolute QUAntitation) peptides with extensive QC for relative and absolute quantitation of proteins.

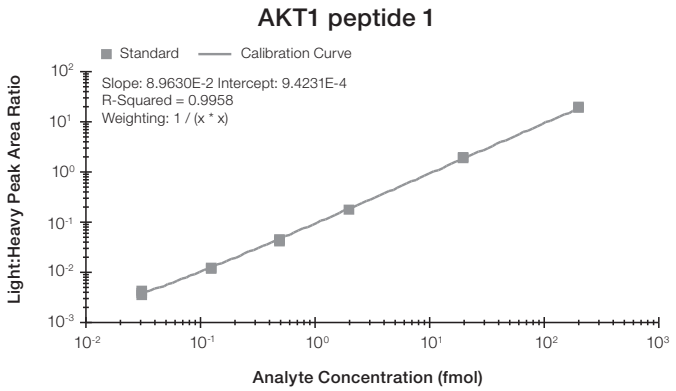
HeavyPeptide and LightPeptide standards are synthesized using the latest Fmoc solid-phase peptide synthesis technology, purified by HPLC and analyzed by mass spectrometry. Amino acid analysis (AAA) provides the most exact peptide concentration to guarantee the highest level of quantitative precision. The different grades of AQUA peptides are defined by the replicates of AAA, with Basic (1X), QuantPro (2X), and Ultimate (3X).

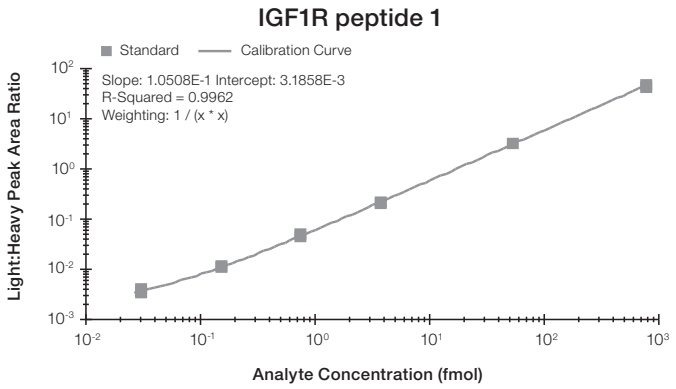
Highlights

- **Precise**—peptide concentration guaranteed from AAA
- **Sensitive**—enables absolute quantification of low-abundance proteins (fmol)
- **Specific**—100% peptide sequence specificity
- **Flexible**—variety of purity, modification, and formatting options

Applications

- Biomarker discovery, verification, and validation
- Functional quantitative proteomics
- Quantitation of posttranslational modifications
- Confirmation of RNA interference (RNAi)
- Pharmacokinetics
- ADME toxicology studies
- Anti-doping testing





Target	Peptide no.	LOD (fmol)	LLOQ (fmol)	ULOQ (fmol)	Linearity (R^2)
AKT1	1	0.008	0.031	200	0.9958
	2	0.008	0.031	200	0.9872
IGF1R	1	0.008	0.031	200	0.9962
	2	0.008	0.125	200	0.9664

Figure 1. HeavyPeptide AQUA analysis. Heavy peptides were selected from discovery MS data. Serial dilutions of light AQUA peptides and constant amounts of heavy AQUA peptides were analyzed in a 6-protein digest matrix using a Thermo Scientific™ Dionex™ UltiMate™ 3000 RSLC Nano System (300 nL/min, C18 reversed-phase column) and Thermo Scientific™ Q Exactive™ HF Hybrid Quadrupole-Orbitrap Mass Spectrometer. Each serial dilution sample was analyzed by targeted PRM-MS. Data were analyzed using Skyline software to generate 6-point calibration curves, and the results for figures of merits are summarized in the table.

Learn more at **thermofisher.com/heavypeptide**

HeavyPeptide and LightPeptide AQUA standards, cont.

Improve quantitative precision for absolute quantitation

Table 6. Specifications of HeavyPeptide and LightPeptide AQUA standards.

	AQUA Ultimate	AQUA QuantPro	AQUA Basic
Research stage	Validation or clinical, ideal for absolute quantitation	Confirmation or validation, ideal for biomarker verification	Discovery, confirmation or validation, relative quantitation
Peptide length*	Up to 15 amino acids		
Amount/number of aliquots	10 nmol/10 aliquots 40 nmol/40 aliquots 96 nmol/96 aliquots	10 nmol/10 aliquots 40 nmol/40 aliquots 96 nmol/96 aliquots	15 to 30 nmol (0.05 to 0.1 mg)/1 aliquot
Purity	>97%	>97%	>95%
Isotopic enrichment	>99%	>99%	>99%
Standard (light) peptides available	Yes	Yes	Yes
Formulation	5 pmol/µL in 5% (v/v) acetonitrile/H_2O	5 pmol/µL in 5% (v/v) acetonitrile/H_2O	Lyophilized
Delivery format	Glass vial		
Production time**	5–7 weeks standard AQUA 2–3 weeks AQUA Express		
Shipment	In solution on wet ice	In solution on wet ice	Dry at room temperature
Quality control	MS, HPLC, quantitative AAA		
Concentration precision	±5–10%	±10–25%	N/A
Modifications	Single or double phosphorylation (pY, pT, or pS) Cysteine carbamidomethylation (CAM) Methionine oxidation [Met(O)] Other modifications available on request		
Optional services	Additional light amino acids to extend peptide length Additional heavy amino acids within the peptide sequence Multiple solvents, concentrations, and aliquot sizes available		

* Please inquire about longer peptide lengths.

** AQUA Express requests are available for up to 10 sequences on a single order and must be approved for express turnaround time.

Table 7. Heavy amino acids offered with HeavyPeptide Custom Synthesis.*

Amino acid	Code	Mass difference	Isotope	Isotopic enrichment
Alanine	A	+4 Da	U-$^{13}C_3$, ^{15}N	>99%
Arginine	R	+10 Da	U-$^{13}C_6$, $^{15}N_4$	>99%
Isoleucine	I	+7 Da	U-$^{13}C_6$, ^{15}N	>99%
Leucine	L	+7 Da	U-$^{13}C_6$, ^{15}N	>99%
Lysine	K	+8 Da	U-$^{13}C_6$, $^{15}N_2$	>99%
Phenylalanine	F	+10 Da	U-$^{13}C_9$, ^{15}N	>99%
Proline	P	+6 Da	U-$^{13}C_5$, ^{15}N	>99%
Valine	V	+6 Da	U-$^{13}C_5$, ^{15}N	>99%

* Other amino acids on request.

PEPotec SRM Peptide Libraries

Fully synthetic, crude peptides customized for the development of medium- to high-throughput SRM and PRM assays

The study of proteomes, subproteomes, and protein pathways often requires quantitative MS analysis that depends on the development and validation of SRM, MRM, and PRM assays. The PEPotec SRM Peptide Libraries offer great convenience and flexibility for the development of quantitative MS with many customizable options.

Highlights

- **Traceable**—peptides are provided in individual 2D barcoded tubes in 96-tube plates
- **Customized**—various grades with optional services available
- **Convenient**—delivered solubilized in 0.1% trifluoroacetic acid (TFA) in 50% (v/v) acetonitrile/water
- **Flexible**—extensive list of available modifications

Applications

- Medium- to high-throughput development of SRM, MRM, and PRM assays
- MS workflows with relative and absolute quantitation strategies

Table 8. PEPotec SRM Peptide Libraries—three grades to fit your experimental needs.

	Grade 1 Fast and easy	Grade 2 Greater analysis	Grade 3 Maximum assurance
Peptide length	6–25 amino acids		
Quantity	≥0.1 mg		
Purity	Crude (as synthesized)		
C-terminal residue*	Lysine (K) or Arginine (R)		
Counterion	TFA		
Formulation	Suspended in 0.1% TFA in 50% (v/v) acetonitrile/water		
Delivery format	Peptides are provided in individual 2-D barcoded tubes in Thermo Scientific Matrix 96-tube plates		
Production time	2–3 weeks		
Shipment	In solution on wet ice		
Minimum order	24 peptides; surcharge for orders of <48 peptides		
Quality control	MS check of 5% of peptides	MS check of 100% of peptides	MS analysis of 100% of peptides
Included documentation	Peptide amount	Peptide amount	Peptide amount and MS spectra
Peptide resynthesis	Not provided	Not provided	One resynthesis provided
Failed synthesis policy	You pay for entire set of peptides ordered	You only pay for peptides successfully synthesized in the requested library	You only pay for peptides successfully synthesized in the requested library
Modifications	Single or double phosphorylation (pY, pT, or pS) Cysteine carbamidomethylation (CAM) Methionine oxidation [Met(O)] Internal heavy amino acids Alternative heavy amino acid at the C-terminus Other modifications available on request		
Optional services	Lyophilization		

* Other stable isotope labeled amino acids are available, please inquire.

Learn more at **thermofisher.com/pepotec-srm**

Learn more at **thermofisher.com/peptides**