



Thermo Scientific Pierce
Custom Peptide Synthesis

standard, modified

heavy peptides and libraries

Thermo
SCIENTIFIC

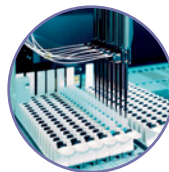
custom peptide synthesis

An overview

Every peptide synthesis is a challenge. At Thermo Fisher Scientific, we have the experience, equipment and knowledge to meet your needs for custom peptide synthesis. Our synthesis team has accumulated tremendous expertise through producing tens of thousands of successful custom peptides. We are constantly adapting our product offering to your needs based on your input. Our expert peptide scientists will support you from the design phase of your peptide to choosing the right modifications, scale or purity for your assay so you achieve the best experimental results for your application.

Thermo Scientific™ Custom Peptide Capabilities:

- Peptides 6-40 amino acids (<6 and up to 75 amino acids also possible)
- Scales from 0.1mg - 1g
- Purities from crude to 98%
- Extensive list of modifications and labels, including heavy peptides
- Flexible formatting options
- Custom conjugation services; e.g., protein-peptide conjugates and multiple antigen peptides (MAPS)



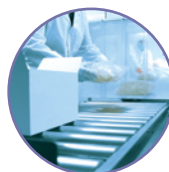
Synthesis

The choice of synthesis platform can affect the cost and/or success of a peptide synthesis. We use several platforms for fully automated Fmoc solid-phase synthesis to provide state-of-the-art products. High-throughput synthesis platforms using 96-well reaction chambers offer longer length and higher purities than spot synthesis platforms. We also offer manual and semi-automated syntheses that usually are better choices for peptides with difficult sequence compositions.



Quality Control

All Thermo Scientific™ Peptides are synthesized in ISO 9001-certified facilities. Quality control for our standard peptide service includes mass spectrometry for identification and analytical HPLC for purity. The detailed spectra is included on the certificate of analysis for your records. Additional quality controls such as amino acid analysis (AAA) and further non-standard analysis are available on request.



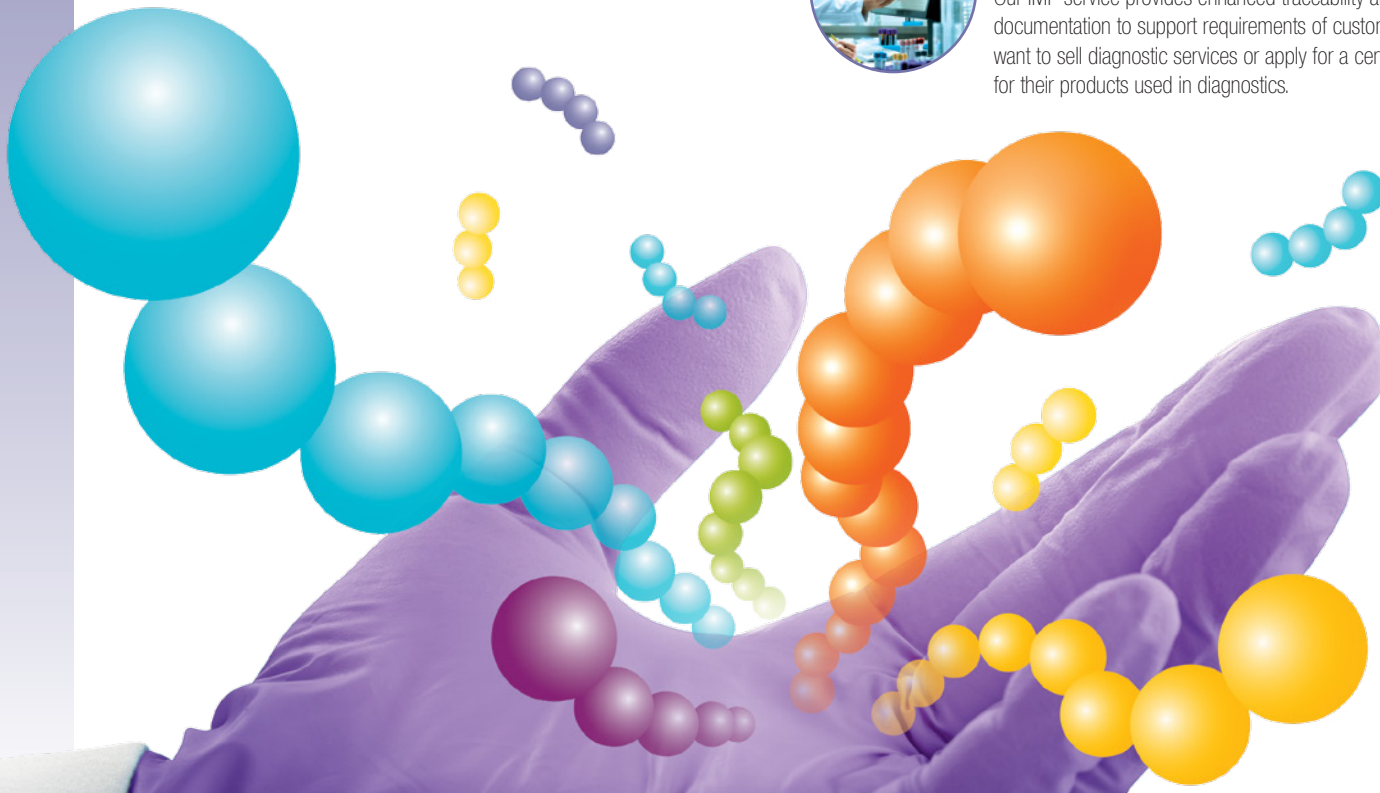
Delivery Format

Unless otherwise stated, our standard peptides are delivered lyophilized with trifluoroacetate (TFA) as a counter ion and packaged in glass vials protected under argon. Exceptions are Thermo Scientific™ Heavy and Light AQUA Kits and our Peptide libraries that are provided in solution and argon-protected. For additional formats, such as delivery in low binding plastics or 2-D barcoded plates or in solution and different solvents, please inquire.



Interactive Manufacturing Practice (IMP) Service

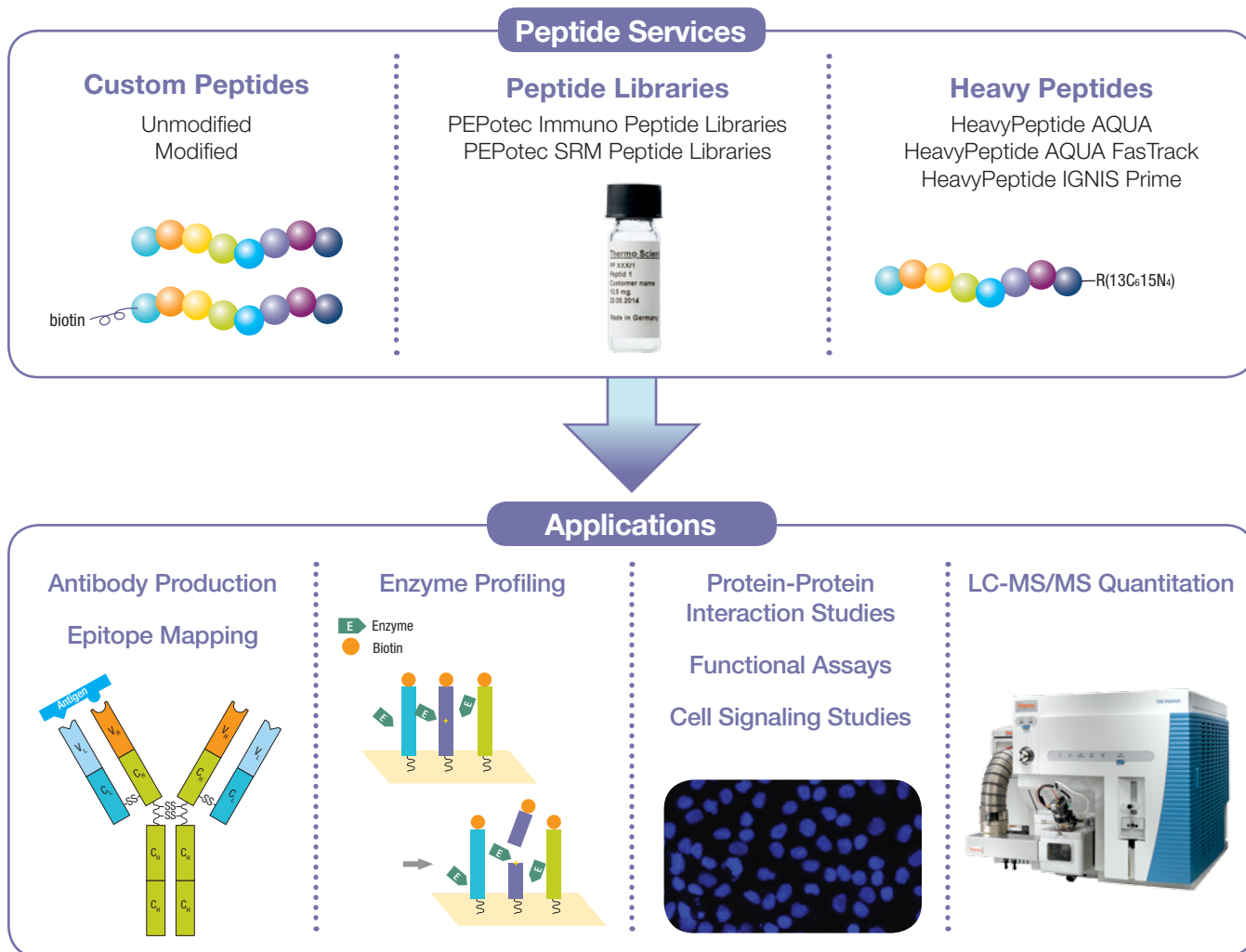
Our IMP service provides enhanced traceability and documentation to support requirements of customers who want to sell diagnostic services or apply for a certification for their products used in diagnostics.



Peptide Applications

The use of synthetic peptides has had a great impact on many areas of research. Because of this variety of research needs, using a supplier that offers a range of peptide services is important to fit your specific applications. Application areas such as antibody therapeutics, epitope mapping and enzyme profiling utilize peptides for antibody production, screening and

assay development. Peptides modified with phosphorylation, acetylation or methylation enable monitoring of cell signaling events. Selection of optimal peptides for antibody or targeted assay development is accomplished through screening of crude peptide libraries. Optimal peptide sequences are then utilized to generate heavy peptide standards for relative and absolute targeted quantitation by mass spectrometry.



custom peptide synthesis

Our validated process delivers high-quality peptides

Custom Peptide Synthesis Service

High-quality peptides tailored to meet your needs.



The Thermo Scientific™ Custom Peptide Synthesis Service offers numerous options for purity levels, modifications and formats for your synthetic peptide order, giving you the flexibility to meet your research needs.

Synthesis is performed using the latest Fmoc solid-phase technology and peptides are purified by HPLC. Peptide

sequence is confirmed by mass spectrometry (MS). Peptides with guaranteed purity are additionally analyzed using analytical HPLC to determine final purity. Peptides are packaged using our ArgonGuard service to minimize amino acid oxidation during shipping and storage. This standard service helps maintain biological activity of custom peptides and reduce experimental variation.

Highlights:

- **High quality** – all peptides are synthesized with high-quality materials and packaged using our ArgonGuard service to help maintain biological activity during shipping and storage.
- **Modifications** – most comprehensive list of available modifications and labels.
- **Validated** – all peptides are analyzed by MS or in combination with analytical HPLC.
- **Flexible** – peptides available in a variety of formats and purities; manual synthesis available for difficult peptides.

Applications:

- Epitope mapping
- Antibody production
- Cell signaling
- Disease research
- Biomarker discovery
- Proteomics
- Enzyme profiling
- Immunization assays

Modifications

We offer a wide range of N-terminal, C-terminal and other modifications with our standard peptide service (Table 2). Please visit thermoscientific.com/pierce for the most up-to-date list and available positions in the peptide sequence.

Table 1. Specifications and available options with Thermo Scientific Custom Peptide Synthesis Service.

Peptide length	4 to 75 [†] amino acids; L- or D-isomers
Manufacturing scale	0.1 to 100mg [‡]
Purity options	From crude to >98% pure
Quality control (QC)	MALDI MS for crude peptides; MALDI MS and analytical HPLC for all other grades of purity
Production options ^{††}	Standard delivery – 2 to 4 weeks Priority production – 1 week

Custom Conjugation Services (6-25 amino acids) are available, including conjugation to carrier proteins (KLH, BSA, OVA or Blue Carrier™ Protein) and multiple antigen peptides (MAPs).

[†] Please inquire about longer peptide lengths

[‡] Greater amounts available upon request

^{††} Depends on region and peptide complexity

Table 2. Peptide modifications offered with standard peptide synthesis.

<ul style="list-style-type: none">• Acetylation of N-terminus (Ac-NH-)• Acetyl-lysine• Aldehyde• Alexa Fluor™ Dyes• 6-amino hexanoic acid (Ahx)• 6-amino caproic acid (Aca)• Amidation of C-terminus (-CONH₂)• Amino benzoic acid• Beta alanine• Bovine serum albumin (BSA)• Biotin• Carbamido methylation• CBZ (Z)• Citrulline• Chloro-L-tyrosine• Conjugation (BSA, KLH)• Conjugation to oligonucleotides• Coumarin• Custom FRET peptides• Cyclization via termini or disulfide bridge• D-amino acids• Dabcyl• Dabsyl• Dansyl• Dihydroxy tyrosine• Dimethyl lysine• Dinitrophenyl (DNP)• DyLight™ Dyes• Dyomics™ Dyes• EDANS• Farnesyl• Fluorescein (FITC/5-FAM)• Formic acid• Glycosylation (N-acetyl Galactose, or Glucose, Mannose)	<ul style="list-style-type: none">• Hydroxy proline• Hydroxy tryptophane• Isotopically labeled amino acids (with ²H, ¹³C, ¹⁵N)• Keyhole limpet hemocyanin (KLH)• Kynurenin• Mercaptopropionic acid• Methoxy-coumarin-acetic acid (MCA)• Methionine sulfone• Methionine sulfoxide [Met(O)]• Monomethyl lysine• Monomethyl arginine• Multiple antigen peptides (MAP)• Myristic acid• 3-nitro tyrosine• Norleucine (Nle)• Octanoic acid• Palmitic acid• Phosphorylation of Ser, Thr and Ty• Polyethyleneglycol spacer (PEG)• Pyro glutamic acid (Pyr)• Rhodamine B• Rhodamine 110• Spacer• Special amino acids (D-amino acids, other amino acids)• Stearic acid• Sulfurylation of Ser, Thr and Tyr• Tetramethylrhodamine (TAMRA)• Texas Red™ Dye• Ubiquitylation• Other dyes or modification on request
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Thermo Scientific 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.

PEPotec Immuno Peptide Libraries are fully customizable and supplied with acetate as the counter ion to avoid potential toxicity issues in epitope discovery and mapping. These application-specific peptide libraries meet the requirements for almost instant use in applications such as vaccine development, T- and B-cell research, antibody development, and biomarker discovery. Optional peptide modifications are available, including residue phosphorylation and acetylation, and different formulations are possible that enable study of cellular signaling events.

Highlights:

- **Low toxicity** – peptides with acetate as the counter ion are less toxic in certain applications when compared with those with trifluoroacetate (TFA)[†].
- **Application-specific** – the 6-20-amino acid peptide length distribution fits most immunological applications.
- **Convenient** – peptides are provided in individual 2-D barcoded tubes in 96-tube plates, and each order includes a CD with the peptide sequences and positions in each plate.
- **Flexible** – optional services, including phosphorylation and acetylation, are available for studying signaling and regulatory proteins.

Applications:

- Epitope mapping of B- and T-cells
- Vaccine development
- Screening of peptide vaccine
- Biomarker discovery
- Cell signalling (kinase/protease studies)

Reference

1. Pini, A. *et al.* (2011). Efficacy and toxicity of the antimicrobial peptide M33 produced with different counter-ions. *Amino Acids* 8 October (epub).



Table 3. Thermo Scientific PEPotec Immuno Peptide Library Standard Service.

Quantity	1-4mg
Length	6 to 20 amino acids; L-isoforms only
Purity[†]	Crude (as synthesized)
QC^{††}	Mass spectrometry (MS), 100% of samples
Formulation	Lyophilized
Vessel	Thermo Scientific™ Matrix™ 96-tube plate format (Product # 3712MTX)
Minimum Order	None, but surcharge for orders of <48 peptides
C-terminus	Any unmodified L-isoform amino acid
Counter-ion	Acetate
Production time	≥8 days (depending on peptide length)
Shipment	Room temperature

[†] No purification given, as peptide purity is sequence-dependent and a function of different liquid chromatographic conditions.

^{††} For all peptides except phosphopeptides, the three major peaks in the MS analysis represent the target of interest. Peptides must pass both the MS analysis and the final gross weight criteria (>1mg) before shipment. If peptides do not pass these criteria, the customer will be informed and one resynthesis will be offered free of charge.

Table 4. Thermo Scientific PEPotec Immuno Peptide Library Optional Services.

Service	Unit Size
Trifluoroacetate (TFA) counter ion	Peptide
Phosphorylation at 1 site	
Phosphorylation at 2 sites	
Lys-Ac, internal	
Peptides >20 and <25-35 amino acids in length	
Delivered in 100% DMSO	Plate
Alphanumeric tube labeling	

Additional modifications upon request.

targeted peptide quantitation

Measuring expression of selected proteins

Whereas discovery proteomics globally profiles and identifies thousands of proteins, targeted proteomics focuses on the quantitation of select proteins and peptides. The end goal of targeted assay development is to quantify selected proteins with high precision, sensitivity, selectivity and throughput. Synthetic peptides are an integral part of targeted assay development. Crude peptide libraries are used as a screening tool, while heavy peptides are utilized for absolute quantitation with selective reaction monitoring (SRM) or multiple reaction monitoring (MRM).

Pharmaceutical and diagnostic applications increasingly rely on quantitative proteomic experiments to quantify proteins in complex samples. Experimental design begins with the software-assisted selection of proteotypic peptide candidates. After synthesis, crude peptides or peptide libraries are screened to identify the best peptide candidates and to optimize the quantitative liquid chromatography-mass spectrometry (LC/MS) assay. After optimal peptide sequence selection, highly pure heavy peptides of the best candidates are then synthesized and purified for target quantitation. The heavy peptides serve as internal quantitative standards for absolute quantification of the corresponding natural

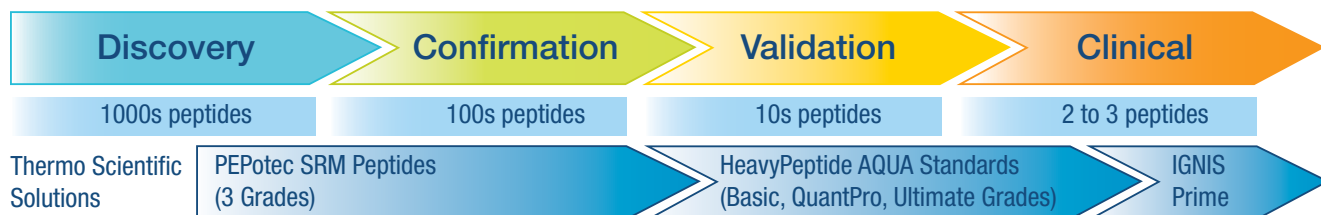
peptides in a biological sample. We offer products that enable assay development from validation to quantitation, including Thermo Scientific™ PEPotec SRM Peptide Libraries and Thermo Scientific™ HeavyPeptide AQUA and IGNIS peptides for quantitation.

Targeted protein quantitation is commonly analyzed with triple quadrupole mass spectrometers, such as the Thermo Scientific™ TSQ Quantiva™ Triple Stage Quadrupole Mass Spectrometer. A triple quadrupole mass spectrometer measures peptides by serially monitoring specific mass windows for peptides of interest, isolating the peptide(s), fragmenting and then quantifying several fragment ions specific for each peptide of interest. This selective reaction monitoring (SRM) strategy for targeted quantitation, along with chromatographic retention time information, provides high sensitivity and specificity. Alternatively, high resolution and accurate mass instruments, such as the Thermo Scientific™ Q Exactive Mass Spectrometer, are being used to quantify proteins with even greater selectivity.

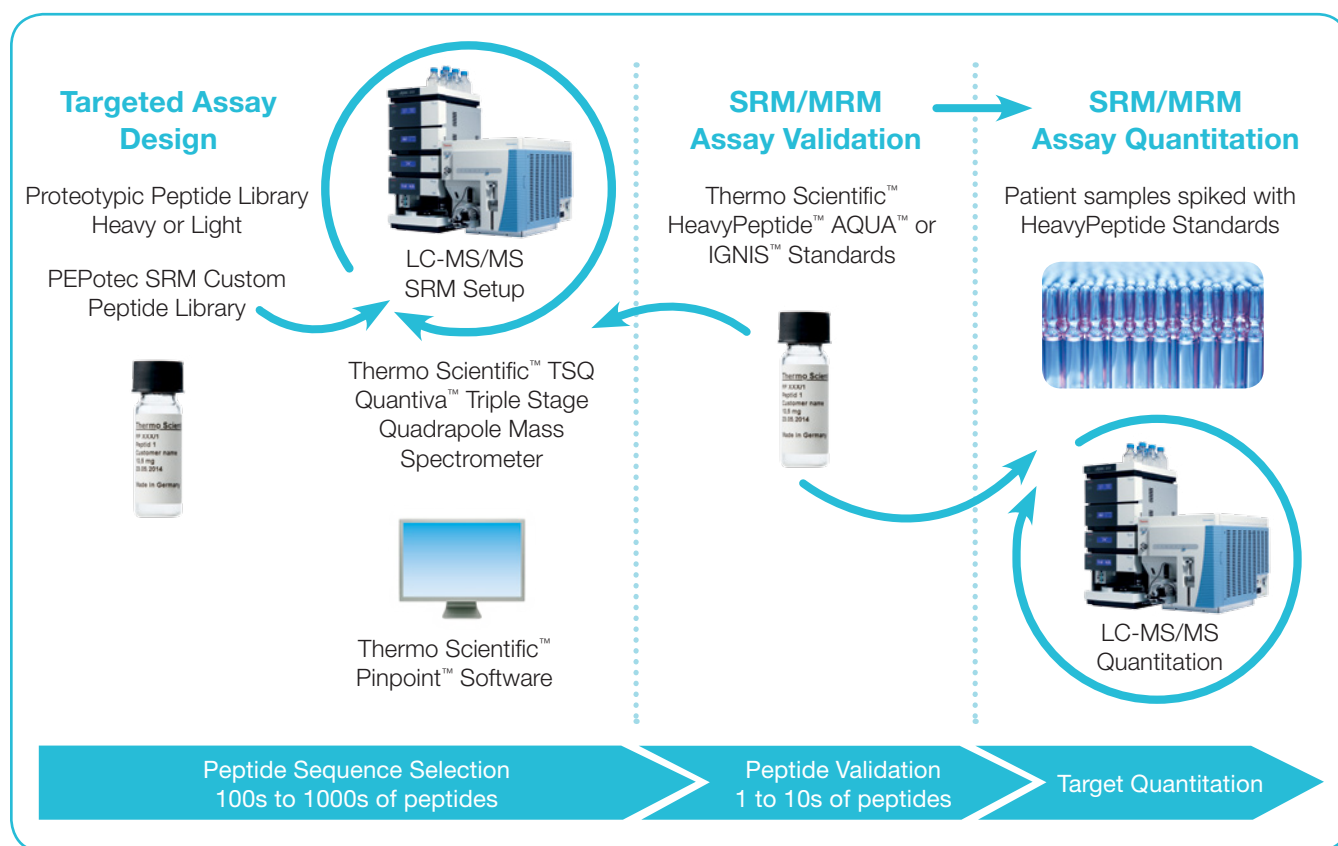
Specialized software such as Thermo Scientific™ Pinpoint™ 1.4 Software ensures as much high-quality data is acquired, and as much valuable information is extracted from that data, as possible.



Targeted SRM Product Summary



Targeted Quantitation Workflow



targeted quantitation with heavy peptides

Measuring expression of selected proteins

Thermo Scientific HeavyPeptide AQUA Standards

High-quality isotopically labelled peptides for absolute quantitation.

The Thermo Scientific™ HeavyPeptide™ AQUA Custom Synthesis Service provides isotopically labelled, AQUA-grade peptides for the relative and absolute quantitation of proteins at very low concentrations in complex protein mixtures.

HeavyPeptide sequences up to 40 amino acids in length are synthesized using the latest Fmoc solid-phase technology, purified by HPLC and analyzed by mass spectrometry. Guaranteed purity of AQUA- (Absolute QUAntitation), Ultimate- and QuantPro-grade peptides is confirmed using stringent analytical HPLC to assure the highest quality peptides for absolute quantitation. We offer advanced heavy peptide synthesis capabilities with a wide range of labels, modifications, scales and purities to meet your research needs. HeavyPeptide AQUA-grade peptides are also part of our Thermo Scientific™ HeavyPeptide™ FasTrack Service to accelerate targeted assay development.

HeavyPeptide Standards are packaged using our ArgonGuard service, in which peptides are packaged in argon gas to minimize amino acid oxidation during shipping and storage. This standard service helps maintain biological activity of custom peptides and reduce experimental variation.

Additionally, Thermo Scientific™ HeavyPeptide™ AQUA FasTrack Service is available to accelerate targeted assay development using selected reaction monitoring (SRM). Highly pure AQUA-, Ultimate- or QuantPro-grade HeavyPeptide Standards are provided in two phases: (1) synthesis of crude peptides for screening and (2) purification of already synthesized peptides for assay development.

Highlights:

- **Accurate** – peptide concentration precision for quantitative application needs.
- **Multiplexed** – up to hundreds of peptides possible.
- **Sensitive** – enables the absolute quantification of low-abundant proteins (fmol).
- **Specific** – 100% peptide sequence specificity.
- **Flexible** – variety of purity, modifications and formatting options.

Applications:

- Biomarker discovery, verification and validation
- Functional quantitative proteomics
- Quantitation of post-translational modifications
- Confirmation of RNA interference (RNAi)
- Pharmacokinetics
- ADME toxicology studies
- Clinical biochemistry for drug and metabolite monitoring
- Anti-doping testing



Table 5. Thermo Scientific HeavyPeptide AQUA Grades.

Grade	Description
AQUA Ultimate	Fully solubilized; concentration precision $\pm 5\%$ *; ideal for absolute quantitation
AQUA QuantPro	Fully solubilized; concentration precision $\pm 25\%$ *; ideal for biomarker verification
AQUA Basic	Lyophilized; relative quantitation

*Depending on sequence composition

Target	LOD (fmol)	LLOQ (fmol)	ULOQ (fmol)	Linearity (R^2)
EGFR	0.7	18.5	1500	0.9999
	0.2	0.7	1500	0.9999
AKT2	0.7	6.2	1500	0.9999
	0.7	6.2	1500	0.9969

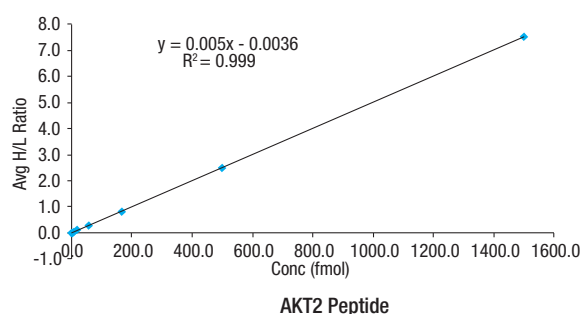
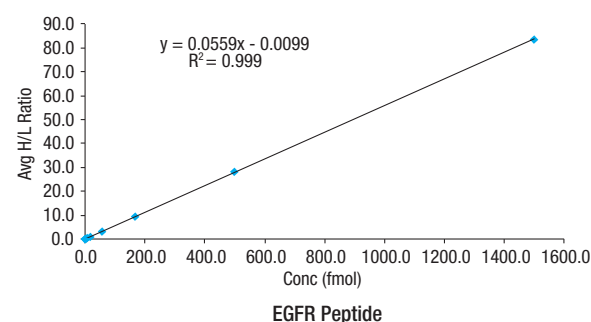


Figure 1. Thermo Scientific HeavyPeptide analysis. Heavy peptides were selected from discovery MS data. AQUA Heavy Peptides were analyzed in a BSA matrix using a Proxeon Easy nanoLC II (300nL/min, C18 reversed phase column) and Thermo Scientific TSQ Vantage Mass Spectrometer. Three transitions were monitored per peptide using the scheduled SRM method. Data was analyzed using Thermo Scientific Pinpoint and Skyline software.

Table 6. Specifications of Thermo Scientific HeavyPeptide AQUA-Grade Standards.

Grade	AQUA Ultimate	AQUA QuantPro	AQUA Basic
Formulation	5pmol/μL in 5% (v/v) acetonitrile/H ₂ O	5pmol/μL in 5% (v/v) acetonitrile/H ₂ O	Lyophilized
Actual concentration	Measured by qAAA [†]	Measured by qAAA [†]	Measured by qAAA [†]
Final concentration	±5-10%*	±10-25%*	NA
Peptide purity	>97%	>97%	>95%
Isotopic enrichment	>99%	>99%	>99%
Peptide length	Up to 30 amino acids	Up to 30 amino acids	Up to 30 amino acids
Amount/No. of aliquots	10nmol/10 aliquots 40nmol/40 aliquots 96nmol/96 aliquots	10nmol/10 aliquots 40nmol/40 aliquots 96nmol/96 aliquots	15 to 30nmol ^{††} (0.05 to 0.1mg)/aliquot
Quality control	MS & analytical HPLC, AAA (±5-10%)	MS & analytical HPLC, AAA (±10-25%)	MS & analytical HPLC
Delivery time [§]	6-8 weeks	6-8 weeks	6-8 weeks
Shipment	In solution on wet ice	In solution on wet ice	Lyophilized at room temp.
Product options	<ul style="list-style-type: none"> • Additional light amino acids to extend the peptide length • Additional heavy amino acid on each peptide • Multiple solvents, concentrations and aliquot sizes available • Peptides delivered in various formats (i.e., 96-well plate with or without detachable tubes in glass or plastic, 2-D barcodes, etc.) 		
Peptide modifications	<ul style="list-style-type: none"> • Single or double phosphorylation (pY, pT or pS) • Cysteine carbamidomethylation (CAM)[‡] • Chloro-L-Tyrosine • Pyroglutamic acid • Methionine oxidation [Met(O)] • Other modifications available on request 		

[†] Quantitative amino acid analysis.

[§] These production times are estimates that vary based on the number of peptides ordered.

^{††} 30nmol is valid for peptides 6-15 amino acids in length. For shorter or longer peptides, the amount might decrease to as little as 15nmol.

[‡] CAM tends to cause cyclisation at the N-terminus. Fully cyclized form can be provided upon request.

* Depending on sequence composition.

Table 7. Heavy amino acids offered with Thermo Scientific HeavyPeptide Custom Synthesis.[†]

Amino acid	Code	Mass difference	Isotope	Isotopic enrichment
Alanine	A	+4Da	U- ¹³ C ₃ , ¹⁵ N	>99%
Arginine	R	+10Da	U- ¹³ C ₆ , ¹⁵ N ₄	>99%
Isoleucine	I	+7Da	U- ¹³ C ₆ , ¹⁵ N	>99%
Leucine	L	+7Da	U- ¹³ C ₆ , ¹⁵ N	>99%
Lysine	K	+8Da	U- ¹³ C ₆ , ¹⁵ N ₂	>99%
Phenylalanine	F	+10Da	U- ¹³ C ₉ , ¹⁵ N	>99%
Proline	P	+6Da	U- ¹³ C ₅ , ¹⁵ N ₂	>99%
Valine	V	+6Da	U- ¹³ C ₅ , ¹⁵ N ₂	>99%

[†] Other amino acids on request.

Table 8. Specifications of Thermo Scientific HeavyPeptide FasTrack Service phases.[†]

Phase	FasTrack 1	FasTrack 2	
Grade	Crude	AQUA Ultimate	AQUA QuantPro
Formulation	Lyophilized	5pmol/μL in 5% (v/v) acetonitrile/H ₂ O	5pmol/μL in 5% (v/v) acetonitrile/H ₂ O
Peptide purity	Crude	>97%	>97%
Amount/No. of aliquots	0.1mg/1 aliquot	10nmol/10 aliquots 40nmol/40 aliquots 96nmol/96 aliquots	10nmol/10 aliquots 40nmol/40 aliquots 96nmol/96 aliquots
Quality control	Mass spectrometry	MALDI MS and analytical HPLC	MALDI MS and analytical HPLC
Delivery time [§]	2 weeks	3 weeks	3 weeks
Shipment	Lyophilized at room temp.	In solution on wet ice	In solution on wet ice

[†] See HeavyPeptide Synthesis Service for all information on HeavyPeptide custom synthesis.

[§] These production times are estimates that vary based on the number of kits ordered.

For a list of references using HeavyPeptide Reagents, please see the back cover.

To order, call 800.874.3723 or 815.968.0747. Outside the U.S., contact your local branch office or distributor.

targeted quantitation with peptide libraries

Measuring expression of selected proteins

Thermo Scientific PEPotec SRM Peptide Libraries

Fully synthetic, crude peptides customized for the development of mid- to high-throughput SRM and MRM assays.

The study of proteomes, sub-proteomes and protein pathways often requires quantitative MS analysis that depends on the development and validation of SRM and MRM assays. Thermo Scientific™ PEPotec™ SRM Peptide Libraries offer great convenience and flexibility for the development of quantitative MS with many customizable options. These peptide libraries were developed as a result of our involvement in the SRMatlas project (peptideatlas.org), which seeks to map the entire human proteome.

The standard service supplies a suspension of at least 0.1mg of each crude peptide housed in individual tubes in a 96-well plate format with either arginine (R) or lysine (K) as the C-terminal amino acid (other C-terminal amino acids are available, too. Contact us for more information.). Three quality control grades are available, and optional services and peptide modifications are offered to give you the peptide libraries that fit your experimental needs.

Highlights:

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



Applications:

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

Includes:

- Fully synthetic crude (as synthesized) peptides
- Multiple grades of QC analysis and optional services and modifications
- Provided in individual Thermo Scientific Matrix 96-Tube plates

Table 9. Thermo Scientific PEPotec SRM Peptide Libraries – Three grades to fit your experimental needs.

	Grade 1 Fast and Easy	Grade 2 Greater Analysis	Grade 3 Maximum Assurance
Quantity	>0.1mg		
Length ¹	6 to 25 amino acids. Up to 35 amino acids are available for an additional fee		
Purity	Crude (as synthesized)		
Formulation ¹	Suspended in 0.1% TFA in 50% (v/v) acetonitrile/water		
Delivery format	Matrix 96-Tube plates (Product # 3712MTX)		
C-terminal residue ¹	R or K		
Counter ion	TFA		
Quality control (QC)	MS check of 5% of peptides	MS check of 100% of peptides	MS analysis of 100% of peptides
Peptide resynthesis ²	Not provided	Not provided	One resynthesis provided
Failed synthesis policy	You pay for entire set of peptides ordered	You pay only for peptides successfully synthesized	You pay only for peptides successfully synthesized
Included documentation	Peptide amount	Peptide amount	Peptide amount and MS spectra
Minimum order ³	24 peptides	4 peptides	4 peptides

¹ Changes to the standard length restrictions, formulation and C-terminal residues are available as optional services.

² Peptides not detected during MS analysis will be resynthesized (depending on the grade selected).

³ Orders for fewer than 48 peptides incur a plate fee.

Table 10. Thermo Scientific PEPotec SRM Peptide Library optional services.

QC: Analytical HPLC & MALDI-MS of 100% of samples*
QC: LC -MS of 100% of samples*
Lyophilized
Individually labeled tubes
Peptides that are 3-5 or 26-35 amino acids in length

* Only for grade 3.

Table 11. Thermo Scientific PEPotec SRM Peptide optional modifications – Available with all grades on a per-peptide basis.

C-terminal heavy labeling at R or K	Acetylation at side chain of Lysine [Lys(Ac)]
Internal heavy labeling at A,R,I,L,K,F,P or V	Methylation at side chain of Lysine [Lys(Me)] and Arginine [Arg(Me)]
Alternative heavy amino acid at C-term	Dimethylation at side chain of Lysine [Lys(Me) ₂]
Alternative light amino acid at C-term	3-Chloro-tyrosine
Phosphorylation at 1–3 sites	Hydroxyproline (Hyp)
All cysteines protected by carbamidomethylation (CAM)	Isoaspartic acid
Diglycine ubiquitination motif on Lysine [Lys(GG)]	Formylation at C-term
Methionine sulfoxide [Met(O)]	CMC - carboxymethylcysteine at cysteine

Others available on request.

Q & A

Q. How are the peptides supplied?

A. Unless otherwise requested, all peptides are delivered as lyophilized trifluoroacetic salts with free amino and carboxy termini. N-terminal acetylation and C-terminal amidation is available for a small fee but must be requested when ordering your peptide(s).

Q. How should peptides be stored?

A. Upon receipt, we recommend preparing single-use aliquots and storing the products immediately at -20°C. In this manner, the lyophilized peptides are stable for several years. Solubilized peptides should be used immediately because they are unstable (the lower the concentration, the more unstable the peptides are). Any remaining peptides in solution should be re-lyophilized for longer storage.

Q. How should peptides be handled?

A. Always wear gloves when working with peptides to avoid contamination (e.g., enzymatic, bacterial, etc.). As many peptides are light-sensitive, they should also be protected from direct light. Avoid repeated freeze-thaw cycles.

Q. How are peptides solubilized?

A. Peptides are complex biomolecules, and each peptide has unique chemical and physical properties because of the unique amino acid composition. Although some peptides are easy to dissolve in aqueous solutions, peptides are often insoluble, especially when they contain long stretches of hydrophobic amino acids.

General guidelines to solubilize peptides:

Because of the unique solubility of each peptide, we **recommend first testing the solubilization of each peptide** with a small amount of product.

1. Always use sterile water or buffer [phosphate buffered saline (PBS), Tris or phosphate, pH 7] to solubilize peptides.
2. Oxygen-free solvents should be used to solubilize peptides containing cysteine, methionine or tryptophan, which are susceptible to rapid oxidation.
3. Allow the peptide to warm to room temperature (preferably in a desiccator) prior to adding the solvent of choice.
4. Solubilization can be improved by warming (<40°C) or sonicating the solution.
5. If the pH of the solution needs to be increased, use only very weak bases to prevent immediate inactivation or racemization.

HeavyPeptide Reagent references

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Guidelines to solubilize hydrophobic peptides:

1. If the product proves insoluble in aqueous buffers due to high hydrophobicity, dissolve a small amount of product in the smallest possible volume of a 50% (v/v) DMSO/water mixture. Then add the desired aqueous solution until the target concentration is achieved.
2. If the product precipitates during this process and cannot be redissolved by adding dimethylsulfoxide (DMSO), then lyophilize the peptide and try again, adding a little more 50% DMSO than the previous attempt.
3. If DMSO interferes with your experimental system, dimethylformamide (DMF) or acetonitrile can serve as alternate solvents.

Q. Can I predict if a peptide is soluble in aqueous solutions?

A. While the amino acid sequence determines the chemical and physical properties of each peptide, we can offer only general guidelines for predicting the solubility of a peptide:

1. Peptides shorter than 5 residues in length are usually soluble in aqueous buffers, except when the entire sequence consists of hydrophobic amino acids (A, W, L, I, F, M, V and Y).
2. Hydrophilic peptides containing >25% charged residues (R, H, K, E and D) and <25% hydrophobic amino acids are usually soluble in aqueous buffers.
3. Hydrophobic peptides containing ≥50% hydrophobic residues may be insoluble or only partly soluble in aqueous solutions. In these cases, we recommend using stronger solvents like DMSO, DMF or acetonitrile.
4. Peptides containing a very high (>75%) percentage of D, E, H, K, N, Q, R, S, T or Y are capable of forming intermolecular hydrogen bonds (crosslinking), thus forming gels in aqueous solutions. These peptides should be solubilized as with hydrophobic peptides (see above).

To minimize solubility problems, optimization of the peptide sequence may be necessary.

Q. What peptide parameters are influenced by amino acid composition?

A. The ability to synthesize the peptide and its solubility, stability, and overall charge. Visit thermoscientific.com/pierce for our **Peptide Design** page in the Protein Methods Library for more information.

For technical help with peptides, please contact services.biopolymers@thermofisher.com

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