thermoscientific

Custom peptide synthesis

Standard | modified | heavy peptides | libraries



Custom peptide synthesis

An overview

Optimizing the efficiency of peptide synthesis can be a challenging task. 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 producing tens of thousands of successful custom peptides. We are constantly adapting our product offering to your needs based on your input. Our experienced peptide scientists will support you from designing your peptide to choosing the right modifications, and to obtain the right scale and/or purity for your assay to help you achieve the best results for your application (Table 1).

Custom peptide synthesis capabilities:

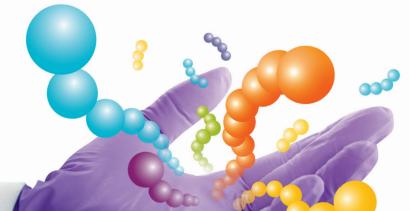
- Thermo Scientific[™] custom peptides 6–40 amino acids (<6 and up to 105 amino acids also possible)
- Scales from 1 mg to 1 g
- Levels of purity from crude to >98%
- Extensive list of modifications and labels, including heavy peptides (isotope-labeled)
- Flexible formatting options
- Custom conjugation services; e.g., protein–peptide conjugates and multiple antigen peptides (MAPs)

Table 1. Specifications and available options with custom peptide synthesis service.

| Peptide length | 4-105* amino acids; L- or D-isoforms |
|----------------------|--|
| Manufacturing scale | 1–1,000 mg** |
| 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 time | Standard delivery within 2-4 weeks |

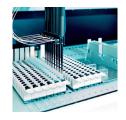
^{*} Please inquire about longer peptide lengths.

^{**} Greater amounts available upon request.









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 peptide synthesis to provide high-quality products. High-throughput synthesis platforms using the 96-well reaction chambers offer longer length and higher purities than the spot synthesis platforms. We also offer manual and semi-automated syntheses that usually are better choices for peptides with difficult sequences.

Quality control

Quality control for our standard peptide service includes mass spectrometry (MS) for identification and analytical high-performance liquid chromatography (HPLC) for determination of purity of the peptides. The detailed spectra are included on the Certificate of Analysis for your records. Additional quality controls such as amino acid analysis (AAA) and further nonstandard analysis are available on request.

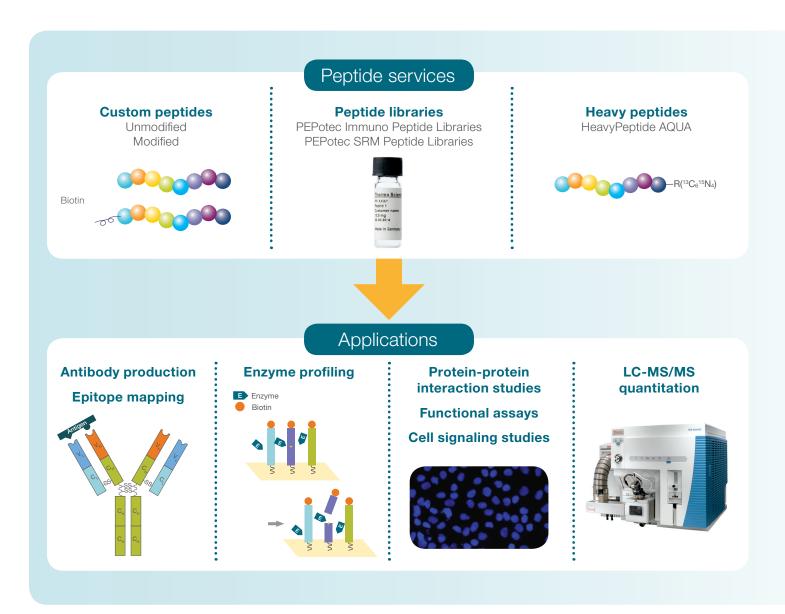
Delivery format

Unless otherwise stated, our standard peptides are delivered lyophilized with trifluoroacetate (TFA) as a counterion and packaged in glass vials, stored under argon to protect from oxygen. The only exceptions are the Thermo Scientific™ Heavy and Light AQUA Kits and our peptide libraries that are provided in-solution and stored under argon. For additional formats, such as delivery in low-binding plastics or 2-D barcoded plates, or in-solution with different solvents, please inquire.

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 one supplier that offers a range of peptide services is important to fit into your specific application. We offer products for antibody therapeutics research, epitope mapping, and enzyme profiling that utilize peptides for antibody production, antibody screening, and assay development. Peptides modified with phosphorylation,

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



Custom peptide synthesis service

High-quality peptides from validated process tailored to help 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.

Peptide synthesis is performed using the latest Fmoc solid-phase technology and peptides are purified by reversed-phase HPLC. Peptide sequence is confirmed by MS. Peptides with guaranteed purity are additionally characterized using analytical HPLC to determine the final purity. Peptides are packaged using our Thermo Scientific™ 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 highquality 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 alone 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 go to **thermofisher.com/peptides** for the most up-to-date list and available positions in the peptide sequence.

Custom conjugation services (6–25 amino acids) are available, including conjugation to carrier proteins (KLH, BSA, OVA, or Thermo Scientific™ Imject™ Blue Carrier™ Protein) and MAPs.

Table 2. 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_a)
- Amino benzoic acid
- Beta-alanine
- Biotin
- Carbamido methylation
- Cbz
- Citrulline
- Chloro-L-tyrosine
- Conjugation to proteins (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)
- Thermo Scientific[™] DyLight[™] dyes
- Dyomics[™] dyes
- EDANS
- Farnesyl
- Fluorescein (FITC/5-FAM)
- Formic acid

- Glycosylation (N-acetyl galactose, or glucose, mannose)
- Hydrocarbon spacers
- Hydroxy proline
- Hydroxy tryptophan
- Isotopically labeled amino acids (with ¹³C, ¹⁵N)
- Kynurenin
- Mercaptopropionic acid
- Methoxy-coumarin-acetic acid (MCA)
- Methionine sulfone
- Methionine sulfoxide [Met(O)]
- Monomethyl lysine
- Monomethyl arginine
- Multiple antigen peptides (MAPs)
- Myristic acid
- 3-nitro tyrosine
- Norleucine (NIe)
- · Octanoic acid
- Palmitoic acid
- Phosophorylation of serine, threonine, and tyrosine
- Polyethylene glycol (PEG) spacer
- Pyroglutamic acid (Pyr)
- Rhodamine B
- Rhodamine 110
- Special amino acids (D-amino acids, other amino acids)
- Stearic acid
- Sulfurylation of serine, threonine, and tyrosine
- Tetramethylrhodamine (TAMRA)
- Invitrogen™ Texas Red™ dye
- Ubiquitination
- Other dyes or modification on request



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 counterion to avoid potential toxicity issues in epitope discovery and mapping (Tables 3 and 4). These application-specific peptide libraries are ready to 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 counterion are less toxic in certain applications when compared with those with trifluoroacetate (TFA)¹
- Application-specific—the 6- to 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
- High-throughput peptide screening
- Biomarker discovery
- Cell signaling (kinase/protease studies)

Table 3. PEPotec Immuno Peptide Library standard service.

| Quantity | 1–4 mg |
|-----------------|---|
| Length | 6-20 amino acids; L-isoforms only |
| Purity* | Crude (as synthesized) |
| QC** | MS, 100% of samples |
| Formulation | Lyophilized |
| Vessel | Thermo Scientific™ Matrix™ 96-tube plate format (Cat. No. 3712) |
| Minimum order | 4; but surcharge for orders of <48 peptides |
| C-terminus | Any unmodified L-isoform amino acid |
| Counterion | Acetate |
| Production time | 3–4 weeks |
| Shipment | Room temperature |
| | |

^{*} No purification given, as peptide purity is sequence-dependent and a function of different liquid chromatographic conditions.

Table 4. PEPotec Immuno Peptide Library optional services.

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

Additional modifications upon request.

Reference

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

^{**} 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 (>1 mg) before shipment. If peptides do not pass these criteria, the customer will be informed and one resynthesis will be offered free of charge.

Targeted quantitation

Measuring expression of selected proteins

Discovery proteomics globally profiles and identifies thousands of proteins, whereas targeted proteomics focuses on the quantitation of selected 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 research 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 from 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™ SRM Peptide Libraries and Thermo Scientific™ HeavyPeptide™ AQUA 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 peptides, 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 helps to ensure the maximum acquisition of high-quality data and extraction of valuable information.



Targeted SRM product summary

Discovery

Confirmation

Validation

1,000s of peptides

100s of peptides

10s of peptides

Solutions

PEPotec SRM peptides (3 grades)

HeavyPeptide AQUA standards (Basic-, QuantPro-, and Ultimate-grades)

Targeted quantitation workflow

Targeted assay design

Proteotypic peptide library Heavy or light

PEPotec SRM Custom Peptide library



TSQ Quantiva triple-stage quadrupole mass spectrometer

LC-MS/MS

SRM setup



Pinpoint software

SRM/MRM assay validation

HeavyPeptide AQUA standards



SRM/MRM assay quantitation

Samples spiked with HeavyPeptide Standards





Target quantitation

Peptide sequence selection 100s–1,000s of peptides

Peptide validation 1–10s of peptides

HeavyPeptide AQUA standards

High-quality, isotopically labeled peptides for absolute quantitation



The HeavyPeptide AQUA Custom Synthesis Service provides isotopically labeled, AQUA (Absolute QUAntitation)-grade peptides for the relative and absolute quantitation of proteins at very low concentrations in complex protein mixtures.

HeavyPeptide standards up to 30 amino acids in length are synthesized using the latest Fmoc solid-phase peptide synthesis technology, purified by HPLC and analyzed by mass spectrometry. Guaranteed purity of AQUA-, 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 help meet your research needs.

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.

Highlights

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

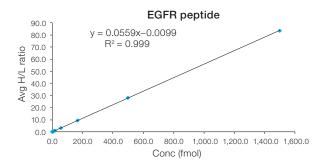
Applications

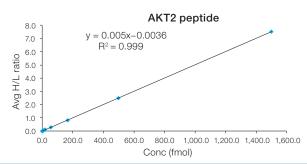
- Biomarker discovery, verification, and validation
- Functional quantitative proteomics
- Quantitation of posttranslational modifications
- Confirmation of RNA interference (RNAi)
- Pharmacokinetics
- ADME toxicology studies
- Anti-doping testing

Table 5. HeavyPeptide AQUA grades.

| Grade | Description |
|------------------|--|
| AQUA Ultimate | Fully solubilized; concentration precision 5–10%;* ideal for absolute quantitation |
| AQUA QuantPro | Fully solubilized; concentration precision 10–25%;* ideal for biomarker verification |
| AQUA Basic | Lyophilized; relative quantitation |

^{*} Depending on sequence composition





| Target | LOD (fmol) | LLOQ (fmol) | ULOQ (fmol) | Linearity (R²) |
|--------|------------|-------------|-------------|----------------|
| EGFR | 0.7 | 18.5 | 1,500 | 0.9999 |
| EGFR | 0.2 | 0.7 | 1,500 | 0.9999 |
| AKT2 | 0.7 | 6.2 | 1,500 | 0.9999 |
| ANIZ | 0.7 | 6.2 | 1,500 | 0.9969 |

Figure 1. HeavyPeptide analysis. Heavy peptides were selected from discovery MS data. HeavyPeptide AQUA peptides were analyzed in a BSA matrix using a Thermo Scientific™ EASY-nLC™ 1200 system (300 nL/min, C18 reversed-phase column) and Thermo Scientific™ TSQ™ Vantage Mass Spectrometer. Three transitions were monitored per peptide using the scheduled SRM method and the results are summarized in the table. Data was analyzed using Thermo Scientific Pinpoint and Skyline software.

Table 6. Specifications of HeavyPeptide AQUA-grade standards.

| Parameters | AQUA Ultimate-grade | | AQUA QuantPro-grade | AQUA Basic-grade |
|----------------------------|---|--|---|---|
| Formulation | 5 pmol/µL in 5% (v/v) acetoni | nitrile/H ₂ O | 5 pmol/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 | 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 [†] (0.05–0.1 mg)/1 aliquot |
| Quality control | MS and analytical HPLC, AA | AA (±5-10%) | MS and analytical HPLC, AAA (±10-25%) | MS and analytical HPLC |
| Delivery time [™] | 3-6 weeks | | 3-6 weeks | 3-6 weeks |
| Shipment | In solution on wet ice | | In solution on wet ice | Lyophilized at room temperature |
| Product options | • / | Additional light amino acids to extend the peptide length Additional heavy amino acid on each peptide Multiple solvents, concentrations, and aliquot sizes available | | |
| Peptide modifications | • (• [•] | 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.

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

| Amino acid | Code | Mass difference | Isotope | Isotopic enrichment |
|---------------|------|-----------------|-------------------------|---------------------|
| Alanine | А | +4 Da | U¹³C₃, ¹⁵N | >99% |
| Arginine | R | +10 Da | U¹³C ₆ , ¹⁵N | >99% |
| Isoleucine | 1 | +7 Da | U¹³C ₆ , ¹⁵N | >99% |
| Leucine | L | +7 Da | U¹³C ₆ , ¹⁵N | >99% |
| Lysine | K | +8 Da | U¹³C ₆ , ¹⁵N | >99% |
| Phenylalanine | F | +10 Da | U¹³C ₉ , ¹⁵N | >99% |
| Proline | Р | +6 Da | U¹³C ₅ , ¹⁵N | >99% |
| Valine | V | +6 Da | U¹³C ₅ , ¹⁵N | >99% |

^{*} Other amino acids on request.



^{**} Depending on sequence composition.

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

^{††} These production times are estimates that vary based on the number of peptides ordered.

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

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. The PEPotec SRM Peptide Libraries offer great convenience and flexibility for the development of quantitative MS with many customizable options.

The standard service supplies a suspension of at least 0.1 mg 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 as well. 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 8. PEPotec SRM Peptide Libraries—three grades to fit your experimental needs.

| Parameters | Grade 1 Fast and easy | Grade 2 Greater analysis | Grade 3 Maximum assurance | | |
|----------------------------|---|--|--|--|--|
| Quantity | >0.1 mg | | | | |
| Length* | 6 to 25 amino acid | ds. Up to 35 amino acids are available fo | or an additional fee | | |
| Purity | Crude (as synthesized) | | | | |
| Formulation* | Suspended in 0.1% TFA in 50% (v/v) acetonitrile/water | | | | |
| Delivery format | Matrix 96-tube plates (Cat. No. 3712) | | | | |
| C-terminal residue* | R or K | | | | |
| Counterion | TFA | | | | |
| Quality control (QC) | MS check of 5% of peptides | MS check of 100% of peptides | MS analysis of 100% of peptides | | |
| Peptide resynthesis** | 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 [†] | 24 peptides | 4 peptides | 4 peptides | | |

^{*} Changes to the standard length restrictions, formulation, and C-terminal residues are available as optional services.

^{**} Peptides not detected during MS analysis will be resynthesized (depending on the grade selected).

[†] Orders for fewer than 48 peptides incur a plate fee.

Table 9. PEPotec SRM Peptide Library optional services.

QC: Analytical HPLC and 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

Table 10. PEPotec SRM Peptide optional modifications—available with all grades on a per-peptide basis.

C-terminal heavy labeling at R or K

Internal heavy labeling at A,R,I,L,K,F,P, or V

Alternative heavy amino acid at C-term

Alternative light amino acid at C-term

Phosphorylation at 1-3 sites

All cysteines protected by carbamidomethylation (CAM)

Diglycine ubiquitination motif on lysine [Lys(GG)]

Methionine sulfoxide [Met(O)]

Acetylation at side chain of lysine [Lys(Ac)]

Methylation at side chain of lysine [Lys(Me)] and arginine [Arg(Me)]

Dimethylation at side chain of lysine [Lys(Me)₂]

3-chloro-tyrosine

Hydroxyproline (Hyp)

Isoaspartic acid

Formylation at C-term

Carboxymethylcysteine (CMC) at cysteine

Others available on request.

Contact us using these email addresses, or to send the quote form.

- Europe, Middle East, and Africa: europeservicespeptides@thermofisher.com
- Rest of world (incl. North America): peps@thermofisher.com

Learn more or download a quote form at thermofisher.com/pepotec



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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 or 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. We recommend preparing single-use aliquots and storing the products immediately at -20°C, upon receipt. 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 freezethaw 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 for solubilizing 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.

^{*} Only for grade 3.

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- 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.

Guidelines for solubilizing hydrophobic peptides:

- 1. If the product proves to be 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 in 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 are discussed in the Protein Biology Learning Center under the Peptide Design section at thermofisher.com/peptidedesign

HeavyPeptide reagent references

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Find out more at thermofisher.com/peptides