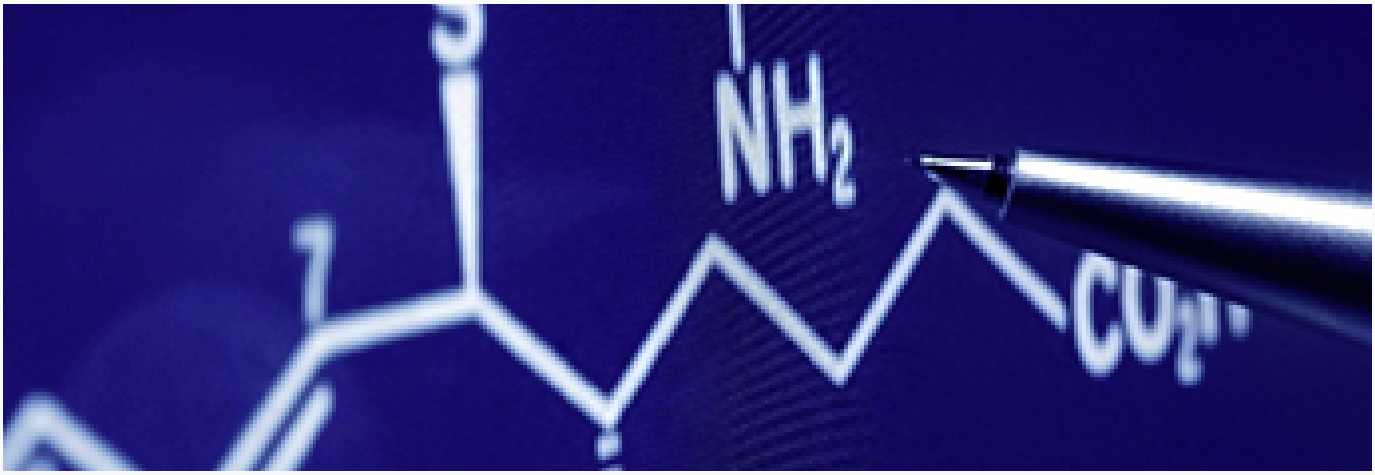
ALTA BIOSCIENCE LTD

Peptide Synthesis

Technical Document

AltaBioscience offers a custom peptide synthesis service certified to ISO 9001. With a strong focus on scientific excellence and 40 years of expertise we work closely with our customers to ensure we meet all their requirements.



Peptides are essential resource tools in areas such as biomedical research, antigen generation, drug development and for the study of physiological and biochemical processes.

Our team of skilled peptide chemists have extensive experience of the range of options available for custom peptide synthesis and will work with you to achieve your goals.

Why use AltaBioscience?

AltaBioscience provides managed solutions helping you save both time and money in your testing and R&D studies. Our instrumentation allows for single batch synthesis of up to 576 small scale peptides in one operation, as well as individual peptides in multigram amounts.

Our HPLC capabilities enables us to offer a range of purification options specific to your applications, from unpurified peptides to purifications of 80-95%.

All purified peptides from AltaBioscience undergo reverse phase HPLC.

- **1mg to multigram amounts**
- **Range of purification options**
- **Cyclic, phosphorylated, fluorescent, isotopic labels, fatty acid incorporation**
- **Peptide Antigen Synthesis and Design Service**
- **Episcan scale: large numbers of small scale peptides suitable for HTS**
- **Histone peptides and microarrays**
- **Custom microarray service**

Advancing science through analytical chemistry and synthesis services in the life and food science sectors worldwide

Synthesis Options and Applications

- **Peptide antigens for antibody generation** Synthesis of peptide to target epitope, incorporation of post-translational modifications into a sequence.
- **Affinity columns for antibody purification** Fast, robust, effective peptide product for the purification of peptide and protein antibodies from serum
- **Linear peptides.** Syntheses from tri-peptides up to 70+ amino acids
- **Branched peptides.** Branching from a C-terminal poly-lysine core or mid-sequence branching. This would enable you to have individual peptides on different branches.
- **Modified amino acid peptides.** Incorporation of D-amino acids, phosphorylated peptides, amino acid analogues, spacers.
- **Tags.** Biotinylated, fatty acids, pegylation, farnysyl, photolabile linkers, maleimide.
- **Fluorescence and dye labelled peptides.** Peptides with fluorescent or other dye labels provide useful tools for monitoring biological interactions, receptor-ligand binding, protein structures and enzyme activity.
- **Isotopically labelled peptides.** For use in quantitative mass spectrometry. Peptides are synthesised incorporating amino acids enriched with the stable isotopes 13C and 15N.
- **Cyclic peptides.** Cyclisation of a peptide provides the benefit of constructing a constrained shaped peptide.
- **Stapled peptides.** Provide stable helical structures, aiding protease resistnace and cell-permeablility and increasing binding affinity to target.
- **Histone peptide synthesis and microarrays.**
- **Custom microarray peptides.** For investigation of protein-peptide interactions using your own peptide sequences.
- **Peptide conjugation**
  - Peptide-protein conjugation KLH, BSA, DNA-hybrids.
  - Peptide -DNA conjugation DNA-hybrids via thiol or amino linkage

Methodology

AltaBioscience uses solid phase synthesis (SPPS) pioneered by Robert Bruce Merrifield, to synthesise peptides. The process involves anchoring the C-terminal of the 1st amino acid to polystyrene based resins then, in a stepwise process, coupling the carboxyl group or C-terminus of the next amino acid to the amino group or N-terminus of another (peptide bond formation). Due to the possibility of unintended side chain reactions, protecting groups are used where applicable.

AltaBioscience uses Fmoc chemistry which enables greater flexibility in synthesis options. Chemical peptide synthesis starts at the C-terminal end of the peptide and ends at the N-terminus.

When the peptide chain is complete, it is cleaved from the resin with acid, a process that also removes the amino acid side chain protection. After removal of the acid, the peptide is ready for QC by HPLC and mass spectrometry before being freeze dried, packaged and dispatched

Peptide Purity

We offer a range of purity options from unpurified peptides through to peptide purities of 80-98%. The purity of all our purified peptides is determined by reverse phase HPLC using either C-18 RP Vydac/Ace or Chromolith columns.

Purity	Use (suitability)
Unpurified peptides	For large numbers of screening grade peptides. For QC, we routinely check ~ 10% of unpurified peptides
80-95%	Standard purity
95-98%	NMR studies, X-ray crystallography analysis and for peptides used as enzyme substrates or for mass spec standards.

Impurities arise from non-target components such as truncated sequences of the target peptide, the peptide with protecting groups still intact, or material used in the cleavage process.

Reverse phase chromatography will remove all the reagents used in the cleavage process.

A wavelength of 215nm is used for the analysis as this is the optimum for the detection of the peptide bond and hence detects all peptide species present. The purity value obtained by this method does not include any water and trifluoroacetate salt which will be present in the dried material. All purified peptides are supplied with HPLC and MS traces. AltaBioscience makes extensive use of capping during synthesis, so deletion peptides are very rare.

Salt form of peptides

AltaBioscience provides various peptide salt forms. Peptides are typically supplied with trifluoroacetate as the counter-ion but we can offer both acetate or chloride salt forms on request.

Peptide purified by HPLC with acetonitrile gradients and trifluoroacetic acid (TFA), exist as their TFA salts but this can be toxic to cell cultures. AltaBioscience can convert the TFA salts to another form to overcome this.

Additional Analysis of Peptides

Additional information about your synthetic peptide provides assurance in the quality of the product synthesised. There are a number of methodologies available from AltaBioscience which will give that added quality assurance.

1. High Performance Liquid Chromatography (HPLC)
HPLC is the primary method of analysing peptide purity. This is performed typically on a C18 reverse phase column, using an acetonitrile water gradient with TFA as the acidic species.

2. Matrix Assisted Laser Desorption and Ionisation (MALDI-TOF)
A 'matrix assisted laser desorption and ionisation – time of flight' mass spectrometer is used to determine the molecular weight of the peptides. Highly accurate, fast and requiring small amounts of sample, it is the ideal method to ascertain that the target peptide has been synthesised.

3. Amino Acid analysis
All dried peptides contain a variable amount of water plus a fixed amount of the peptide counter-ion, usually TFA. Quantitative amino acid analysis is the only method which enables the net peptide content to be determined. The peptide is acid hydrolysed to its amino acids and these are quantified after separation by ion exchange chromatography and detection with ninhydrin. Here, the amount of each amino acid is measured after total acid hydrolysis, the sum total of which gives the amount of peptide in the product. Typical values for net peptide content range from 70 - 90%.

4. N-terminal Sequencing
Amino N-terminal Edman sequencing can be used to confirm the correct sequence order of amino acids.

Design and structure of peptides

AltaBioscience can incorporate all the standard amino acids and other moieties from the extensive range of building blocks available. We only use high quality reagents sourced from reputable suppliers to ensure our customers receive products of high quality. By convention, peptides are written left to right with the N-terminus at the left and the C-terminus at the right. Synthetic difficulty is sequence dependent however our peptide chemists, using their experience in synthesis optimisation, have increased our success rate of synthesising difficult peptides e.g. amyloid peptides and proteins.

Amino acid classification

The following table gives a general of the amino acids

Class	Amino Acid
Acidic, polar	Asp, Glu His, Lys, Arg Asn,
Basic, polar	Cys, Gly, Gln, Pro, Ser, Thr,
Polar uncharged	Tyr Ala, Ile, Leu, Met, Phe,
uncharged	Trp, Val
Nonpolar and hydrophobic	

Length of peptides

Our synthesis methods are suitable for peptides and proteins in the range of 3-70 amino acids. AltaBioscience has experience in successfully making peptides of over 80 amino acids. The longer the peptide, the greater the number of impurities to be removed from the target sequence, thereby affecting the absolute purity of the product. A longer peptide will also have a higher chance of containing a sequence region that is difficult to synthesise, however with many years of experience AltaBioscience has overcome such challenging peptides. We can advise on any possible concerns regarding the viability of a synthesis.

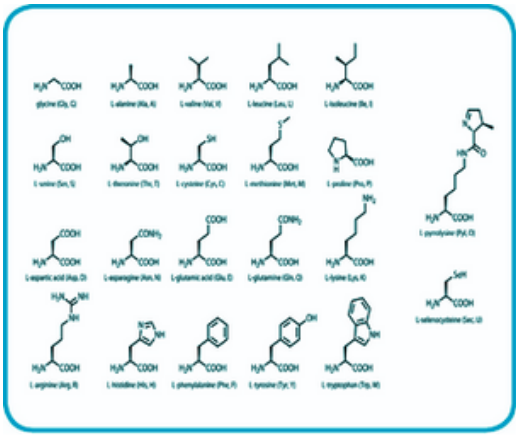


Image showing structure of amino acids

Design considerations

The following may be helpful when determining peptide design.

- Proline breaks up beta sheet formations and although non-polar, helps to solubilise peptides.
- A spacer between a dye or tag and the rest of the peptide sequence can be advantageous.
- It is always more cost effective to put a dye or tag at the N-terminus rather than the C-terminus as this can be incorporated during the standard synthesis.
- N-terminal glutamine (Gln) should be avoided. It is very unstable and rapidly forms the cyclic pyro glutamic acid. It is best to add either pyroglutamic acid itself or include an acetyl group at the N-terminal glutamine.
- Avoid regions containing long strings of valine or isoleucine as these are extremely difficult to incorporate and may prevent any extension of the synthesis.
- Peptides with no charged or polar groups may be very insoluble. These amino acids decrease solubility: - Trp, Val, Ile, Phe.
  These amino acids increase solubility: - Lys, His, Arg, Asp, Glu, Ser, Thr.
- Multiple additions of phospho-amino acids can sometimes be difficult to incorporate during synthesis.
- Naturally cysteine occurs in proteins with a disulphide bridge, therefore we would advise to avoid cysteine when designing peptides for raising antibodies.

Modifications and unnatural amino acids

A number of modifications are possible; the most commonly encountered are listed below.

- Norleucine (Nle)
- 6-Aminohexanoic acid
- Aminobutyric acid (Abu)
- β-alanine
- Aminobenzoic acid
- Citrulline
- Ornithine
- ε-Acetyl-lysine
- Hydroxyproline
- Mercaptopropionic acid (MPA)
- 3-Nitro-tyrosine

Phosphorylated amino acids
Phosphorylated Ser ,Thr and Tyr can be placed at any specified site in a peptide. However, their incorporation should be limited so as to not cause any synthesis or purification problems.

Terminus modifications
N-terminal acetyl and C-terminal amides remove the charges at the ends of a peptide which mimics the parent protein.

Methylation
Mono, di and tri methylated Lys, mono and dimethyl Arg are found in histone proteins, these methylated amino acids can be easily incorporated at positions.

D amino acids
All the D amino acids can be added at any position.

Analogues
As amino acids determine shape, fine tuning the shape of peptides can be advantageous. AltaBioscience can incorporate analogues enabling longer or shorter versions of the side chain length of some amino acids. For example, homoserine and homoarginine are longer variants of serine and arginine while ornithine and diamino butyric acid are shorter analogues of lysine.

Isotopes
Amino acids enriched with the stable isotopes 13C and 15N can be incorporated into peptides for use in quantitative mass spectrometry. It is advised to focus on the amino acids with nonreactive side chains, such as Val and Phe.

Unnatural amino acids
Compounds such as phenylglycine, naphthyl alanine, nor-leucine and beta-alanine are readily incorporated into peptides.

Spacers

These are used to pull dyes and tags away from the active site of a peptide. Typical examples are shown here, others are available on request:

- **Hydrophobic, aminohexanoic acid**
- **Hydrophilic, SGSG a short peptide sequence**
- **Hydrophilic PEG, ranging from 9 to 88 atoms (these can aid solubility**)

Biotin
This compound binds irreversibly to streptavidin a property which enables the immobilisation of peptides onto different platforms. It is used extensively in screening assays and to bind peptide to substrates

Desthiobiotin
This compound binds to streptavidin but can be displaced by biotin. It has the advantage of releasing the peptide after any binding studies.

Peptide Types

Cyclic peptides
AltaBioscience can synthesise both cyclic and cross linked peptides.

Cyclic disulphide
If a peptide is made with two cysteine residues, careful oxidation in solution will result in a cyclic compound. The bridge can be broken under physiological conditions.

Cyclisation via amide bond
Using amide bond formation, stable cyclisation can be achieved. This can be via the terminal ends of the peptide or alternatively, where the sequence allows, using suitable residues mid-sequence resulting in a cyclic peptide.

Cyclic thioethers
These are useful when designing peptide libraries where the peptide needs to be presented as a constrained shape. The thioether bond is stable under physiological conditions.

Cross linking peptides
Many bioactive peptides contain several disulphide bridges. AltaBioscience has had considerable success in the synthesis of these complex compounds.

Peptides with Dyes

A very wide range of dyes and tags are available for incorporation into the peptide. These peptides are used for monitoring biological interactions, receptor-ligand – binding, protein structures and enzyme activity.
Typical examples are shown here, others are available on request:

	Absorption (nm)	Emission (nm)	Alternative
Mca	340	405	Alexa 350
Edans	335	493	Dylight 405
Dansyl	324	518	Dylight 350
Dabcyl	400	550	FAM, TAMIRA
FAM	495	518	FITC, Alexa Dyes
NBD	465	540	FAM, Alexa Dyes
TAMRA	556	588	Cy3

Stapled peptides

Short fragments of proteins and peptides lack helical structures, easily undergo proteolysis degradation and have difficulty in cell permeation. These difficulties can be overcome by hydrocarbon-staple modification of peptides, providing stable helical structures, relatively protease resistance, cell-permeable and bind with increased binding affinity to their target. Hydrocarbon stapling may provide a useful strategy in researching experimental and therapeutic modulation of protein-protein interactions as well as in vivo pharmacokinetics studies.

Histone peptides and microarrays

Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes, acting as spools around which DNA winds, and play a role in gene regulation. AltaBioscience has a range of options for investigations in histone-protein interactions.

Peptides for custom micro arrays

AltaBioscience can synthesise peptides and using printing technology, immobilise them onto slides or 96-well plates. Peptides for custom micro arrays are commonly used for epitope mapping and screening of peptide vaccines.

To reduce steric hindrance effects, it is helpful to incorporate a spacer such as Ahx or a PEG between the peptide sequence and any biotin which is used to anchor the peptide onto the array slide. The biotin is usually added at the N-terminus but we can incorporate it at the C-terminus or anywhere along the peptide chain.

Peptides for antibody generation

In general, synthetic peptides are too small to elicit an immunogenic response. Peptide antigens can be coupled onto a carrier protein or synthesised onto a larger molecule to ensure that it will be large enough to be detected by the immune system.

AltaBioscience offers 3 options for peptide antigen syntheses.

1. Multiple Antigenic Peptides (MAPs)

MAP peptides are octomeric molecules with peptide chains branching out from a central poly-lysine core. The eight peptide chains increase the molecular weight of the compound sufficiently for it to be easily recognised as an antigen. It provides an easy and flexible method for antibody production and is a method best suited for regions away from the C-terminus of a protein.
It is also possible to make chimeric MAPs containing two different peptide sequences, each forming four of the chains of the 8-branched structure. This option could provide a method of presenting 2 peptide antigens in a single inoculation.

Optional purification for MAP peptides is through a 2-3kDa membrane.

In addition to antigen synthesis we can also simultaneously synthesise an affinity column for use in antibody purification.

2. PEG-Peptide Antigens

An alternative to MAPs, provide a prolonged residence in body, a decreased degradation by metabolic enzymes and a reduction or elimination of protein immunogenicity.

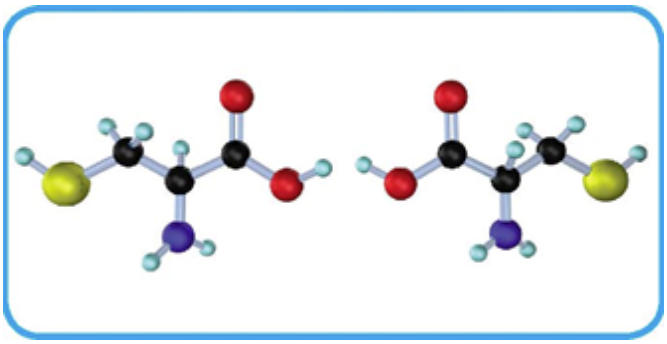
3. Peptide – protein conjugates

By conjugating KLH, BSA or other suitable proteins via a linker molecule, peptides can be prepared for use in antibody generation. The size of the protein triggers the antibody system, which recognises the attached peptides. The most popular carrier proteins are BSA (bovine serum albumin) and KLH (keyhole limpet haemocyanin), which elicits a strong antibody response and contains a very large number of lysine residues which are used to attach the peptide antigen.

This particular approach can be used to attach the peptide in any orientation, i.e. at either the N or the C terminus. However, it is not suitable for peptides containing cysteine, as that amino acid is added to the sequence to act as the linker to the protein.

Peptide antigen design considerations

Our chemists have considerable experience in peptide antigen design and will work with you to achieve the best chance of success. In general, peptides for antibodies will be hydrophilic and flexible coming from the exterior of the parent protein. A hydophilicity plot will indicate which parts of the protein are likely to be on the outside of the structure. The Kyte-Doolittle or the Hopp-Woods algorithms will be very useful here. Structure predictions can be done with Chou-Fasman plots. Peptide epitope regions containing cysteine should be avoided where possible as these in nature would present a very different shape to the immune system compared with the disulphide bridged cystine.



Storage

All our peptides are supplied as freeze dried materials which are regarded as stable compounds for shipping purposes.

For long term storage however, it is recommended to store them at -20°C. When taking them out of the freezer, it is important to allow the bottles/vials to warm up to room temperature before opening the container as peptides are often hygroscopic and it avoids condensation of atmospheric water on the peptide.

Peptides in solution can degrade, primarily due to oxidation of Cys, Met and Trp residues but they are also susceptible to attack by microbes, so it is advised to store solutions at -20°C when not in use.

If preferred, we can supply your peptide aliquoted into smaller amounts for ease of use.

Solubility

Peptide solubility is determined not only by sequence but by the peptide environment. In general, peptides with a large proportion of nonpolar amino acids will be difficult to dissolve in aqueous solutions, the more polar residues that are present, the easier it will be to dissolve a peptide. Peptides that are acidic, i.e. contain more acidic amino acids than basic, will be more soluble at higher pH and peptides that are overall basic will be most soluble at lower pH.

Dissolving peptides

We recommend the use of volatile materials such as dilute acetic acid and ammonia solutions when first dissolving an unknown peptide. This will enable the buffers to be removed by lyophilisation ant the dissolution attempted again if necessary.

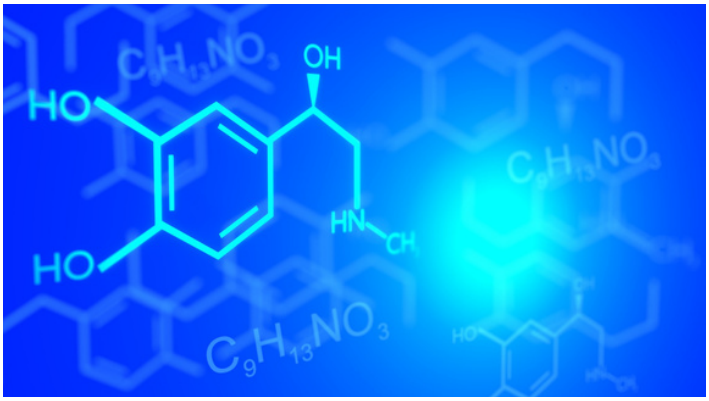
Suggestions

- If the peptide is acidic, i.e. contains more Asp and Glu residues than His, Lys or Arg, dissolve the peptide in dilute ammonia solution, e.g. 0.5% ammonium hydroxide. Do not use this method if your peptide has disulphide bridges, the high pH may cause them to unfold.
- If the peptide is basic, i.e. contains an excess of His, Lys and Arg groups, the n 10% acetic acid may be suitable to dissolve the peptide.
- DMSO is a very good solvent and has the advantage of being tolerated by cells, it is however difficult to remove by drying. One method is to add a small amount of high purity grade DMSO to the stock peptide solution until it dissolves. Once dissolved, water or buffer solution can be added very slowly to dilute the DMSO content. Stop the water addition if the peptide starts to precipitate out. **DMSO is not suitable for peptides containing single cysteines as it promotes disulphide bridge formation.**
- Gentle warming and sonication are useful tactics in getting peptides to dissolve.

Peptides originating from the trans-membrane regions of proteins are generally very difficult to dissolve

You will receive:

A datasheet containing the peptide sequence, Purity Quantity and details of modifications. Traces of mass spec and HPLC Chromatograms are also provided for purified peptides.



Safety

All peptides supplied are for research use only. Toxicological properties have not been investigated, thus appropriate precautions must be taken to avoid skin and eye contact, ingestion and inhalation.

About AltaBioscience

AltaBioscience, was founded in 1973, originally within The University of Birmingham. The ISO 9001 certified company provides services for academic research and for the pharmaceutical, biotechnology and healthcare industries.

CONTACT US

For more details on custom peptide synthesis, visit
www.altabioscience.com

To speak to one of our senior peptide chemists, please contact us by telephone on **+44 (0)1527 584495**
or email us at **info@altabioscience.com**

Advancing science through analytical chemistry and synthesis services in the life and food science sectors worldwide