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## Custom Peptide Synthesis - FAQs

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### 1. What is the typical turn-around time for peptide synthesis?

Our typical turn-around time is 2-3 weeks for standard peptide under 30 amino acids. The turn-around time varies depending on the peptide length, solubility and difficulty.

### 2. How do you ship peptides? What QC data will be provided?

All peptides will be shipped in lyophilized powder. Each peptide comes with CoA, MS data and HPLC data.

### 3. Which purity is recommended for my application?

Here is our recommended guideline:

>75%, preferably >85%: immunological applications, polyclonal antibody production and nonsensitive screening

>90%: SAR studies, bioassays

>95%: In vitro bioassays such as ELISA, enzymology, biological activity

>98%: Structural studies such as Crystallography, NMR or sensitive bioassays.

### 4. Peptide has TFA salt form and Acetate salt form, which form I shall choose?

By default, peptide is synthesized in TFA form. For cell or animal research, you shall consider having peptides produced in acetate form at 98% or higher to avoid abnormal responses *in vivo*. Acetate salt form can be requested at additional cost.

### 5. What kind of terminal choice is appropriate?

By default, chemically synthesized peptides have free amine at N terminal and free acid at C terminal. N-terminal acetylation and C-terminal amidation are uncharged, which reduce the overall charge of a peptide so that the solubility may decrease. However the modifications are desirable since it imitates its natural structure. It increases the metabolic stability of the peptides and their ability to resist enzymatic degradation by aminopetidases, exopeptidases, and synthetase. This enhances their ability to enter cells, thus may increase the biological activity of a peptide.

We recommend the modifications for intracellular, in-vivo assays and in-vitro functional studies. The modified peptides can then be used as substrates in enzyme assays. Amidation not only enhances the activity of peptide hormones, it also prolongs their shelf life. The modifications can reduce the influence of charged C- or N-terminal during ELISA binding assays.

### 6. How to dissolve my peptide?

Upon delivery, we will include a **Peptide Handling Guideline** which will help you in dissolving your peptide properly. You may request peptide solubility test at additional cost. Feel free to contact our technical support if you need further assistance. If you are really concerned about solubility, you may request peptide solubility test at additional cost at the time of ordering.

### 7. How do I store my synthetic peptides?

Most lyophilized peptides will be stable at room temperature for at least a few weeks. For long-term storage, you shall store lyophilized peptides at -20°C. Repeated freeze-thaw cycles should be avoided. Allow to come to room temperature before opening. The shelf life of peptide solutions is limited; a peptide solution once prepared should be used as soon as possible.

8. **What synthetic methods do you use? Is there any limitation on the length of peptides that can be achieved?**  
For peptides with less than 50 amino acids, we use chemical method to do the synthesis. For peptides with more than 50 amino acids, we use custom recombinant method. Fmoc solid-phase peptide synthesis is employed to build linear peptides. Peptide is synthesized from its C-terminus by stepwise addition of amino acids. Initially, the first Fmoc-amino acid is attached to an insoluble support resin via an acid labile linker. After de-protection of Fmoc by treatment with piperidine, the second Fmoc-amino acid is coupled utilizing a pre-activated species or in situ activation. After the desired peptide is synthesized, the resin bound peptide is de-protected and detached from the resin via TFA cleavage.
9. **What if some problems come up during the synthesis or purification process?**  
Each peptide has its specific characteristics. If some problems happen during the synthesis beyond our expectation, and we may not deliver your peptide on time, we will inform you as soon as possible. By slight chance that we are not able to make the peptide, you will not be charged for any cost.
10. **What is the method of Fluorescein labeling? What is the difference between Fluorescein and FITC?**  
FITC (Fluorescein isothiocyanate) is activated precursor used for the Fluorescein labeling. For the efficient N-terminal labeling, a seven-atom aminohexanoyl spacer (NH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-CH<sub>2</sub>-COOH) is inserted between the fluorophore (fluorescein) and the N-terminus of the peptide. This spacer helps to separate the fluorophore from its point of attachment, potentially reducing the interaction of the fluorophore with the biomolecule to which it is conjugated and making it more accessible to secondary detection reagents.
11. **Is C-terminal labeling of Biotin (or FITC) possible?**  
Yes. C-terminal labeling of Biotin (or FITC) is done by addition of a Lys residue at the C-terminus of a peptide, and Biotin (or FITC) is attached to the lysine side chain via amide bond. Lysine's positive charge is removed.
12. **What is the appropriate peptide length for antibody production?**  
Generally, 10–20 (mostly 15) residue peptide is recommended. Longer peptide could have more epitopes, but could have more chance to form stable secondary structure which is not native form. Shorter peptide is generally not good unless there are valid reasons for it, such as potential sequence homology with a related family member or other proteins.
13. **Should I consider adding a Cysteine in peptides for making antibodies?**  
Chemical conjugation using Cysteine offers a single point attachment provided there is just one Cys in the sequence (added or part of the native sequence). It is preferable to add Cys at the NH<sub>2</sub> terminus if the peptide is internal or it represents the very C-terminus. This will keep the COOH free (non-conjugated) as it exists in native protein. For peptides representing the very NH<sub>2</sub>-terminal sequences, Cys should be added at the C-terminus of the peptide. For internal peptides, Cys can be added at either end but it is easier to synthesize peptides containing a NH<sub>2</sub>-terminal Cysteine. Cysteine can also be used to couple peptides to Sepharose for affinity purification of antibodies. Amino or COOH-conjugation chemistries should be avoided as most peptides contain several NH<sub>2</sub> and COOH groups available in a given peptide sequence resulting into multi-point attachment and peptide distortion.
14. **Conjugation method?**  
In order to generate immune response, peptides should be conjugated to bigger carrier proteins. You have a choice of BSA, ovalbumin, or KLH. One of the advantages of KLH (keyhole limpet hemocyanin) is that it does not interfere with ELISA or Western Blot because it is not used for blocking reagent. General conjugation method is maleimide method which couples the Cysteine residue of the peptide to the carrier protein. For the peptide conjugation, one Cysteine residue should be added to N- or C-terminus of the peptide in order to be linked to carrier protein. Please select a less important terminus to be conjugated to protein. If your peptide is N-terminal sequence of a protein, addition of Cysteine to C-terminus will be good, and vice versa. If your peptide has internal Cysteine residue, C-terminal conjugation via the carboxyl group by EDC method is used when there is no Glu, Asp residues, or N-terminal

conjugation via amino group by Glutaraldehyde method is used when there is no Lys residue.

**15. What is a MAP?**

MAPS or Multi-Antigenic Peptide is a branched peptide at which linear peptide chains are linked at their C-terminus via polylysine core, thereby increasing the size of whole molecule. This is done to eliminate the coupling of peptides to KLH. It seems that, however, conformation of peptides on MAP is less flexible, and antibodies obtained by MAP often recognize protein less often than by conventional KLH conjugation. In addition, there is no free peptide produced when making MAP, making it difficult to remove polylysine core directed antibodies. Purification of MAP by HPLC is difficult, and MAP is provided without mass verification due to its heterogeneity and large molecular size.

**16. Why does my KLH/Peptide solution appear cloudy?**

KLH or Keyhole Limpet Hemocyanin is a large aggregating protein ( $MW = 4 \times 10^5 - 1 \times 10^7$ ). Because of its size and structure, its solubility in water is limited, causing a cloudy appearance. This shall not affect immunogenicity and the turbid solution can be used for immunizations.

**17. What is the purity for the crude peptide and the desalted peptide? How do you purify the peptide? What are the impurities?**

For short peptides with normal sequences under 15aa, it is generally 40-60% by HPLC for crude grade; 50-70% by HPLC for desalted grade. The longer peptide is, the lower purity is for crude or desalted.

Peptides are generally purified by HPLC using water and acetonitrile gradient. Most impurities are incorrectly synthesized peptides, such as fragments or deletion peptides, incompletely de-protected peptides and residual water.

**18. Can you explain the M+Na and M+K mass peaks in MALDI spectra?**

It is very common to see Na (sodium) and K (potassium) adducts in the MALDI spectrum. The sodium and potassium comes from the water used in the peptide solvents. Even distilled and deionized water has trace amounts of sodium and potassium ions, which can never be entirely removed. These become ionized during the MALDI mass spec process and bind to the free carboxyl groups of the peptide. Because there is no water purification system that will remove every single sodium or potassium ion from water, seeing the sodium and potassium adducts at times is very common and unavoidable in MALDI mass spec. This is not an indication that the peptide is not pure, nor should it be confused with an incorrect molecular weight.

**19. Explanation of peptide purity, peptide content and absolute amount of desired peptide.**

Biomatik ships peptides according to the actual gross weight of the lyophilized product. This product includes any impurities such as fragments or deletion peptides, incompletely de-protected peptides, TFA, and residual water.

**Peptide purity** measured by HPLC. It is the amount of correct peptide relative to all analytes that absorb at ~214 nm (the peptide bond absorbs), most likely deletion, truncation or incompletely deprotected sequences, etc. Peptide purity does not take into account water and salts that are usually present in the sample.

**Peptide content** is the percentage of total peptides present in the product relative everything else present in the sample, such as water, salts, etc. Peptide content is determined by nitrogen content analysis.

**Absolute amount of desired peptide** in the lyophilized peptide powder thus can be concluded by the formula: The peptide content X the peptide purity.