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Custom Peptide Synthesis - FAQs

1. **Do you have customer testimonials and publications citing your peptide synthesis service?**
   We do collect customer testimonials and you can find some testimonials on our web pages. Our support team will be happy to send the testimonials upon your request. You can check out Google Scholar for some of the publications citing our peptide synthesis service: http://scholar.google.com

2. **What is the typical turn-around time for peptide synthesis? How do you ship and how long does it take to ship to me?**
   Our typical turn-around time is 2-3 weeks for a standard peptide under 30 amino acids. The turn-around time varies depending on the peptide length, solubility and difficulty. All peptides will be shipped in lyophilized powder, at room temperature. It is overnight delivery within USA/Canada, and takes typically 3-4 days to reach researchers in other countries.

3. **Which analytical data do you provide for peptides?**
   Peptide name and sequence
   Lot number
   Molecular formula
   Theoretical molecular mass
   Molecular mass, obtained by mass spectrometry
   Purity, determined by HPLC
   Appearance
   Solubility test (upon request)

4. **Which purity is recommended for my application?**
   >75%, preferably >85%: immunological applications, polyclonal antibody production and non-sensitive 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

5. **TFA salt form vs. Acetate or HCl salt form: Which form should I choose?**
   By default, peptides are synthesized in TFA salt. For cell or tissue culture related experiments, you should consider having peptides produced in acetate or HCl salt form at 98% or higher to avoid abnormal responses. Acetate or HCl salt form can be requested at additional cost. Please note, switching to acetate salt or HCl salt does not reveal nor guarantee TFA salt level in the final product.

6. **How long you can synthesize a peptide?**
   Biomatik can synthesize peptides up to 130aa. Unlike many peptide suppliers who are only comfortable in making peptides under 30 or 40aa, Biomatik has extensive experience in making peptides ranging from 40aa to 90aa. The success rate is much lower when it comes to longer peptides, especially 100aa or longer. If you plan to synthesize a sequence of 130aa or longer, please contact us for custom protein expression and purification service.

7. **What type of end terminal modification 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 reduces the overall charge of a peptide so the solubility may decrease. However, the modifications are desirable since it imitates its natural structure. It increases the metabolic stability of peptides and their ability to resist enzymatic degradation by aminopeptidases, exopeptidases, and synthetases. This enhances their ability to enter cells, thus increasing 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.

8. **How to dissolve my peptide?**
   Upon delivery, we will include a **Peptide Handling Guideline** which will help you in dissolving your peptide properly. You can also download a copy from our website. You may request peptide solubility test at the time of ordering, free of charge.

9. **How do I store my synthetic peptides?**
   Most lyophilized peptides will be stable at room temperature for 2-3 weeks. For long-term storage, you should 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.

10. **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 cannot deliver your peptide on time, we will inform you as soon as possible. By chance that we are not able to make the peptide, you will not be charged for any costs.

11. **What is the method of Fluorescein labelling?**
    FITC (Fluorescein isothiocyanate) is the activated precursor used for the Fluorescein labelling. For the efficient N-terminal labelling, a seven-atom aminohexanoyl spacer (NH2-CH2-CH2-CH2-CH2-CH2-COOH) is inserted between the fluorophore (fluoroscein) 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.

12. **Is C-terminal labelling of Biotin (or FITC) possible?**
    Yes. C-terminal labelling of Biotin (or FITC) is done by addition of a Lysine residue at the C-terminus of a peptide, and Biotin (or FITC) is attached to the Lysine side chain via an amide bond. Lysine's positive charge is removed.

13. **What is the appropriate peptide length for antibody production?**
    Generally, a 10-25 residue peptide is recommended. A longer peptide could have more epitopes, but could also have a greater chance of forming stable secondary structures which are not native forms. A shorter peptide (<10aa) is generally not good unless there are valid reasons for it, such as potential sequence homology with a related family member or other proteins.

14. **Should I consider adding a Cysteine in my peptide for carrier protein conjugation for antibody production?**
    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). If your peptide does not have an existing Cys in the sequence, it is preferable to add Cys at the NH2 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 the native protein. For peptides representing the very NH2-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 NH2-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 NH2 and COOH groups available in a given peptide sequence which can result in forming multiple attachment points or peptide distortion.

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 typically recognize target protein less often than by conventional KLH conjugation. In addition, there is no free peptide produced when making MAPS, making it difficult to remove polylysine core directed antibodies. Purification of MAPS by HPLC is difficult, and MAPS is provided without mass verification due do its heterogeneity and large molecular size.

16. **Why does my KLH-conjugated peptide solution appear cloudy?**
    KLH or Keyhole Limpet Hemocyanin is a large aggregating protein (MW = $4x10^5$ – $1x10^7$). 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 and 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 the peptide, the lower the purity for crude or desalted.
    Peptides are generally purified by HPLC using water and acetonitrile gradient. Most impurities are fragments or deletion peptides, incompletely de-protected peptides, and residual salt and 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. **Which peptide analytical services do you provide?**
    Mass spectrometry (MS) is an analytical tool used for measuring the molecular mass of a sample. MS analytical data is included in each peptide delivery from Biomatik.

    HPLC Purity is the amount of target peptide relative to the total amount of material that absorb at ~220 nm (the peptide bond absorbs) i.e. the desired target peptide and other fragment peptides. Peptide purity by HPLC does not take into account water and salts that are usually present in the sample, since water and salts do not absorb at ~220 nm. HPLC analytical data is included in each peptide delivery from Biomatik.

    Net Peptide Content is the percentage of all peptides (the desired target peptide and other fragment peptides) present relative to everything (including the target peptide, other fragment peptides, salts and water) present in the lyophilized peptide powder. Salts and water do not contain nitrogen, thus the net peptide content can be determined by nitrogen elemental analysis.

    Along with HPLC Purity, Net Peptide Content can be used to measure the net weight of target peptide in the lyophilized peptide powder. This information is important when calculating the concentration of target peptide during sensitive experiments.

TFA Content: As a standard in the industry, all of our peptides are delivered in TFA salt. TFA content analysis can be performed if you would like to know the actual TFA salt content in the lyophilized peptide powder. If you are engaged in cell or animal related research, you should consider having your peptide delivered in acetate or HCl salt.

KF Test (Water Content Test): Karl Fischer titration is a classic titration method to determine trace amounts of water in a sample.

20. **How to determine net weight and molar amount of the desired target peptide?**
Biomatik ships peptides according to the gross weight of lyophilized powder. The lyophilized powder includes impurities such as fragment peptides, salts and residual water.

Net weight of desired peptide: Gross Weight X Net Peptide Content (%) X HPLC Purity (%)
Molar amount of desired peptide: Net Weight / Molecular Weight

An example: Peptide sample, gross weight 10 mg, with the following analytical data:
HPLC Purity 98.5%, Net Peptide Content 86.0%. Mol. weight 1360.65 g/mol.

According to the above formulas:
Net Weight of desired peptide: 10 mg X 98.5% X 86.0% = 8.471 mg
Molar Amount of desired peptide: 8.471 mg / 1360.65 g/mol X 1000 = 6.226 µmol

The above value allows calculating concentrations, when dissolving the peptide.