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Peptide Synthesis FAQs

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Below are some frequently asked questions about the Biomatik Peptide Synthesis service. If you have any other questions, please contact us at peptide@biomatik.com.

1. Do you have customer testimonials and publications citing your peptide synthesis service?
Biomatik has been trusted among top scientists and researchers worldwide for quality products and custom services since 2002. To date, Biomatik has delivered 70,000+ custom-made products to our customers worldwide. Learn why Biomatik has been trusted by thousands of researchers worldwide by viewing our customer testimonials, selected publications , and 4,000+ publications citing our fine products and services at Google Scholar.

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 turnaround time is 2-3 weeks for a standard purified peptide under 30 amino acids. The turnaround time varies depending on the peptide length, solubility, and difficulty. Turnaround Time (TAT) indicated on the quote is an estimated time only. It does not reflect any production difficulties, if so encountered. All peptides will be shipped in lyophilized powder, at room temperature. It is overnight delivery within the USA/Canada, and typically takes 3-4 days to reach researchers in other countries.

3. Which analytical data do you provide for peptides?
By default, each peptide comes with CoA, MS spectra, and HPLC chromatograms. See Sample QA Report. We can provide additional analysis if required, e.g. Peptide content analysis, TFA content analysis, and KF test (Water content test).

4. How do I design custom peptide sequences for my applications?
The sequence, amino acid composition, and length of a peptide will influence whether correct assembly and purification are feasible. These factors also determine the solubility of the final product. Please refer to our Peptide Design Guideline on some factors in the design of a peptide sequence for synthesis.

5. Which purity is recommended for my application?
Please refer to our Recommended Peptide Purity Guideline.

6. TFA salt form vs. Acetate or HCl salt form: Which form should I choose?
By default, all research peptides are synthesized in TFA salt form. For cell-based assays or animal studies, you should consider having TFA salt removed by switching it to acetate or HCl salt form (with TFA <1% guarantee) to avoid abnormal responses. Depending on the budget, you may also want to consider higher purities (>98%) to get optimal results.

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7. How long can you 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. However, the success rate is getting 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.

8. 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 the shelf life. The modifications can reduce the influence of charged C- or N-terminal during ELISA binding assays.

9. How to dissolve my peptide?
Peptide solubility information is included on the CoA if you have requested a solubility test at the time of ordering, free of charge. If it is not available, you can follow a Peptide Handling Guideline.

10. 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. For more details, please refer to Peptide Handling Guideline.

11. 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 - which is our "No Peptide, No Charge" policy.

12. Is C-terminal labeling of Biotin (or FITC) possible?
Yes. C-terminal labeling of Biotin (or FITC) is done by the addition of a Lysine residue at the C-terminus

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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. Biomatik also offers custom antibody production service, please contact us for more details or check out our Antibody Production Service page for information.

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 the whole molecule. This is done to eliminate the coupling of peptides to KLH. It seems that, however, the 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 to 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 of the crude and desalted peptide? How do you purify the peptide? What are the impurities?

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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 common to see Na (sodium) and K (potassium) adducts in the MALDI spectrum. The sodium and potassium come 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 no water purification system will remove every single sodium or potassium ion from water, seeing the sodium and potassium adducts at times is 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. How about batch-to-batch consistency with research grade custom peptides?
Research grade custom peptides come with limited quality control (MS and HPLC analysis only). Therefore, the variation is expected from batch to batch in terms of the net peptide content of the desired target peptide, salt content, water content, solubility, etc. Such batch-to-batch variation shall not create an issue for most experiments.

However, if you are engaged in sensitive bioassays and you want to minimize batch-to-batch variation or determine the exact peptide dosage used in the experiments, you shall request additional quality control and analysis (e.g., peptide content, salt content, water content, solubility), at an additional cost.

For example, if you are engaged in cell related assays, you shall request TFA removal (<1%); If you must apply the same peptide dosage for your experiment from batch to batch, you shall require peptide content analysis; If you need high solubility or concentration (e.g. 5mg/ml) in a specific solvent from batch to batch, you shall ask for solubility control and testing. For the ultimate batch-to-batch consistency, consider GMP-grade peptides.

20. Which peptide analytical services do you provide?

- MS Analysis: By default, MS analytical data is included in each peptide delivery from Biomatik. Mass spectrometry (MS) is an analytical tool used for measuring the molecular mass of a sample.
- HPLC Analysis: By default, HPLC 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 absorbs 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.

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- Peptide Content Analysis: Also called “Nitrogen Elemental Analysis”. Peptide content analysis is the percentage of all peptides (the desired target peptide and other fragment peptides) present in the powder relative to everything (including the desired target peptide, other fragment peptides, salts and water) present in the powder. Salts and water do not contain nitrogen, thus they are not considered in the analysis. Along with HPLC purity, net peptide content can be used to measure the net peptide weight of the desired target peptide in the lyophilized peptide powder. This information can be important to determine peptide dosage for some sensitive experiments.
- TFA Content Analysis: By default, research 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-based assays and animal studies, you shall consider having your peptide produced in acetate or HCl salt.
- KF Test (Water Content Analysis): Water Content Analysis can be requested at an additional cost. Karl Fischer titration is a classic titration method to determine trace amounts of water in a sample.

21. How to determine net peptide weight, net peptide concentration, or net peptide molar?
By default, research grade peptides are shipped according to the gross weight of lyophilized powder and come with MS and HPLC analytical data only. The lyophilized peptide powder includes the desired target peptide, other peptide fragments, and impurities such as salts, water, etc.

“Net Peptide Weight” refers to the net weight of the desired target peptide. Peptide Content Analysis is used to determine the net peptide content of the desired target peptide. Peptide content is the percentage of all peptides (the desired target peptide and other fragment peptides) present in the powder relative to everything (including the desired target peptide, other fragment peptides, salts, and water) present in the powder. Along with HPLC purity, net peptide content is used to measure the net peptide weight of the desired target peptide in the lyophilized peptide powder.

With the net peptide weight, we can then calculate net peptide concentration or net peptide molar:

- Net Peptide Weight (Weight of the desired peptide) = Gross Weight (mg) X HPLC Purity (%) X Net Peptide Content (%)
- Net Peptide Concentration (Peptide concentration of the desired peptide) = Net Peptide Weight (mg) / Volume (ml)
- Net Peptide Molar (Molar amount of the desired peptide) = Net Weight (mg) / Molecular Weight

Calculation example:
Peptide “A1”:
Gross weight is 10 mg, HPLC purity 98.5%, Molecular weight 1360.65 g/mol.
Net peptide content (per Peptide Content Analysis): 86.0%

- Net Peptide Weight = 10 mg X 98.5% X 86.0% = 8.471 mg
- Net Peptide Concentration (1mg gross weight in 1ml solvent): 0.8471 mg/ml
- Net Peptide Molar = 8.471 mg / 1360.65 g/mol X 1000 = 6.226 µmol

Notes: We don’t typically recommend but for peptides containing W or Y residues, net peptide concentration can also be determined based on the extinction coefficient of W or Y residues (Absorbance at 280 nm).