Biomatik
Tel: (519) 489-7195, (800) 836-8089
Fax: (519) 231-0140, (877) 221-3515
Email: info@biomatik.com
http://www.biomatik.com

Peptide Handling (Solubility & Storage) Guideline

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How to Dissolve a Peptide?

Peptide solubility is sequence dependent and it is primarily determined by its overall charge. Based on our experience, over 70% of peptides can be dissolved in water, while almost 99% of peptides can be dissolved in DMSO. The solubility information can be found on the Certificate of Analysis. If the solubility information is not known, please follow this solubility guideline.

Step #1 – Determine the Overall Charge

Acidic residues (negatively charged)	D, E, and the C-terminal free acid COOH	Charge: -1
Basic residues (positively charged)	R, K, H, and the N-terminal free amine NH2	Charge: +1
Hydrophobic uncharged residues	F, I, L, M, V, W, and Y	Charge: 0
Uncharged residues	G, A, S, T, C, N, Q, P, acetyl, and amide	Charge: 0
Examples: EFIRKRHDGASDL: (+5) + (-4) = +1. This is a positively charged peptide (basic peptide). Try Method #1 below. EHRLGAEKDEFIS: (+4) + (-5) = -1. This is a negatively charged peptide (acidic peptide). Try Method #2 below. EFISEHRLDAGAK: (+4) + (-4) = 0. This is a neutral peptide. Try Method #3 below.		

Step #2 – Choose the Proper Dissolution Method

Do NOT use the whole batch for solubility test. We recommend 1mg aliquot for solubility testing purpose.

Method #1 – For Basic Peptides (Overall Charge >0):
Try to dissolve a small aliquot (1mg is recommended) with sterile water first. Sonication can be tried to enhance the solubility. If it fails, try a few drops of 10% - 30% acetic acid. If it fails as well, add trifluoroacetic acid [TFA] (<50µl) to fully dissolve the peptide, and then dilute with sterile water or buffer to the desired concentration. *Note: Do not try TFA if the peptides are for cell culture and assays. In this case, try Method #3 below.

Method #2 – For Acidic Peptides (Overall Charge <0):
Try to dissolve a small aliquot (1mg is recommended) with sterile water or 1X PBS (pH 7.4) first. Sonication can be tried to enhance the solubility. If it fails, add a small amount of NH4OH (<50µl) or 10% ammonium bicarbonate (dropwise) to fully dissolve the peptide, and then dilute with sterile water or buffer to the desired concentration. Ensure that the resulting pH of the peptide solution is about 7 and adjust the pH if needed. *Note: Do not try ammonium hydroxide for Cys-containing peptides as it may lead to disulfide bond formation. In this case, try Method #3 below.

Method #3 – For Neutral Peptides (Overall Charge =0) or Highly Hydrophobic Peptides:
Try to dissolve a small aliquot (1mg is recommended) with a small amount of DMSO. Once the peptide is completely dissolved in DMSO, add it slowly (dropwise) to sterile water or buffer to the desired concentration.

The use of DMSO is discouraged for peptides containing cysteine as it may oxidize the side-chain functionalities. In such cases, use DMF or acetonitrile (ACN) instead.

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- Final concentration of 5% DMSO (v/v) is recommended for most experiments except for cell assays.
- 0.5-1% DMSO is "generally safe" for most cell assays. Some cell assays may tolerate up to 5% DMSO, while some others may only tolerate 0.5% DMSO or lower. It is recommended to maintain as low DMSO concentration as possible for the cell assays.

  Note: You may prepare the stock solution with higher DMSO concentration and dilute it with sterile water or buffer to the desired concentration. If aggregation is observed (which may happen to some peptides containing cysteines), add a small amount of 8M urea or 6M guanidium-HCL to the peptide, then proceed with the above dilution.

If the solubilization fails, the peptide solution can be lyophilized and another attempt of solubilization can be tried.

How to Store a Peptide?

Lyophilized peptides are shipped at ambient temperature and most peptides are stable at room temperature for a couple of weeks or more. For long term storage, lyophilized peptides shall be stored at -20°C or colder, away from bright light.

The stability of each peptide depends on its sequence and structure. We suggest storing lyophilized peptides at -20 °C. It is recommended that peptides containing methionine, cysteine, or tryptophan residues be stored in an oxygen-free atmosphere to avoid oxidation. The presence of dithiothreitol (DTT) can be useful in preventing oxidation.

Exposure to moisture will decrease long-term stability of lyophilized peptides. Therefore, before taking an aliquot from a peptide tube, allow the peptide to warm up to room temperature before opening. This will reduce the uptake of moisture that is present in the surrounding atmosphere.

A peptide solution once prepared should be used as soon as possible. If peptide storage in solution is unavoidable, we recommend that you store it in aliquots at -20°C (preferably −80°C). Repeated freeze-thaw cycles should be avoided. Use a 0.2 µm filter to remove bacterial contamination if needed.

The shelf life of peptide solutions is limited, especially for peptides containing cysteine (C), methionine (M), tryptophan (W), asparagine (N), glutamine (Q), or N-terminal glutamic acid (E). For example, a Cys-containing peptide is easily oxidised, especially in basic conditions; some residues are easy to racemise, such as proline. Peptide solution stability becomes worse if the pH is 8 or higher. Therefore, we recommend keeping stock solutions in the range of pH 4-6.