Peptide Solubility Guidelines

Often times a challenging part of working with synthetic peptides is determining the best solvent in which the peptide will dissolve. Although some peptides easily dissolve in aqueous solutions, a common problem encountered is very low solubility or even insolubility, especially peptides with long sequences of hydrophobic amino acids. Known characteristics of individual amino acids (Table 1) can help to predict a peptide's solubility. The guidelines below may be helpful in preparing peptide solutions.

General Guidelines for Preparing Peptide Solutions

For best results, test peptide solubility with a small amount of product. Allow the peptide to warm to room temperature (preferably in a desiccator) before reconstituting. Always use sterile water or buffer (Tris or phosphate, buffer at pH 7) when preparing solutions. For peptides containing Cys, Met or Trp, which are susceptible to rapid oxidation, use oxygen-free solvents. Solubility also can be facilitated by warming ($< 40°C$) or sonication. When increasing the pH of the solution, use only weak bases to prevent immediate inactivation by racemization or side reactions.

Peptides shorter than five residues are usually soluble in water or aqueous buffer, except when the entire sequence consists of hydrophobic amino acids (e.g., W, L, I, F, M, V, Y).

Hydrophilic peptides containing > 25% charged residues (e.g., D, K, R, H and E) and 25% hydrophobic amino acids are usually soluble in water or aqueous buffers. If the peptide is basic [i.e., basic residues are more than acidic residues in the peptide] and does not dissolve in water, try 10% and higher solutions of acetic acid, and dilute with sterile water to the desired concentration. If the peptide still does not dissolve, add TFA (<50ul) to dissolve the peptide and dilute to the desired concentration with sterile water. If the peptide is acidic [i.e., acidic residues are more than basic residues in the peptide] and does not dissolve in water, add a small amount of base such as NH4OH (<50ul) or 10% ammonium bicarbonate to dissolve the peptide and dilute to the desired concentration with sterile water.

If the peptide tends to aggregate (usually peptides containing cysteines), add 6 M urea, 6 M urea with 20% acetic acid, or 6 M guanidine•HCl to the peptide, then proceed with the necessary dilutions.

Additional Guidelines for Hydrophobic Peptides

Peptides containing 50% and more hydrophobic residues might be insoluble or only partly soluble in aqueous solutions. In this case, dissolve the peptide in the smallest possible volume of 50% (v/v) DMSO, DMF or ACN(acetonitrile) in water and subsequently add water or buffer until the desired concentration is reached. If the peptide precipitates again during this process and cannot be re-dissolved by adding

DMSO, lyophilization and another attempt of solubilization will be required. DMSO is the preferred solvent for hydrophobic peptides when working in cell culture because of its low toxicity. If DMSO interferes with your experimental system, use DMF or ACN as an alternative. Acetonitrile is the referred solvent in ESI-LC/MS but is highly volatile, which can result in difficulties during handling and determining concentration. For best results, maintain the ACN concentration below 50%.

In addition, peptide containing a high proportion (> 75%) of D, E, H, K, N, Q, R, S, T, Y are capable of building intermolecular hydrogen bonds (i.e., crosslinking), thus forming gels in aqueous solutions. Treat these peptides according to the instructions for hydrophobic peptides or change the pH value, if possible. If the peptide is not soluble in solvents compatible with your biological assay, optimization of the peptide sequence is necessary. Shortening the sequence or adding hydrophilic or charged amino acids results in higher solubility.

Table 1. Amino acid characteristics

Peptide Solubility	
Hydrophilic	D, E, H, K, N, Q, R, S, T, hydroxy-proline, pyro-glutamic acid
Intermediate	C, G
Hydrophobic	A, F, I, L, M, P, V, W, Y, alpha-amino butyric acid, b-amino alanine, norleucine
Peptide Stability	
Oxidation in relatively mild conditions	C. M
De-amidation, dehydration, cyclization to pGlu	N, Q, C-terminal amides, N-terminal Q
Degradation during peptide preparation	M, W
Charged residues	
Positive	K, R, H, peptide N-terminus
Negative	D, E, Y, peptide C-terminus