Peptide Handling Guideline

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Proper peptide solubilization is the starting point of a successful bioassay project. Always use a small aliquot of your peptide to test the reconstitution conditions. The solubility of a peptide is primarily determined by its polarity (overall charge).

How to Calculate the Overall Charge of a Peptide?

a) Assign a value of -1 to each acidic residue: D, E, and the C-terminal free acid (-COOH);
b) Assign a value of +1 to each basic residue: R, K, H, and the N-terminal free amine ($-NH_2$);
c) Hydrophobic uncharged residues: F, I, L, M, V, W, and Y;
d) Uncharged residues: G, A, S, T, C, N, Q, P, acetyl, and amide.

Examples:
EFIRKRHDGASDL: (+5) + (-4) = +1. This is a positively charged peptide (basic peptide).
EHRLGAEKDEFIS: (+4) + (-5) = -1. This is a negatively charged peptide (acidic peptide).
EFISEHRLDAGAK: (+4) + (-4) = 0. This is a neutral peptide.

How to Dissolve a Peptide?

Note: The concentration of a stock peptide solution is recommended at around 1mg/ml.

1) A positively charged peptide (basic peptide) can be dissolved in water or acidic solutions. Try to dissolve the peptide in water first. If the peptide persists as visible particles, sonication can be tried. If it fails, try a few drops of acetic acid (10-25%). If it fails as well, add a very small amount of Trifluoroacetic acid [TFA] (<50µl), and then dilute it with water to your desired concentration. Note that TFA is not recommended for cell-based assays and animal studies.

2) A negatively charged peptide (acidic peptide) can be dissolved in water or basic solutions.
Try to dissolve the peptide in water or PBS (pH 7.4) first. If the peptide persists as visible particles, sonication can be tried. If it fails, add a few drops of 0.1M ammonium hydroxide (<50µl) then dilute it with water to your desired concentration. For peptides that contain Cys, do not try basic solutions as it may lead to disulfide bond formation. Try the method 4) below.

3) A neutral peptide can normally be dissolved in organic solvents. Try adding a small amount of acetonitrile, methanol, or isopropanol until the peptide is fully dissolved, and then dilute it with water or your buffer to the desired concentration.

4) For a very hydrophobic peptide (which contains high portion of hydrophobic residue, while the overall charge can be positive, negative or neutral), try to dissolve it by adding a few drops of DMSO until the peptide is fully dissolved, and then dilute it with water to the desired concentration. For peptides that contain Cys, use DMF instead of DMSO.

   For peptides that tend to aggregate, add a few drops of 6M Guanidine HCl, or 8M Urea, and then proceed with the above reconstitution guideline.

How to Store a Peptide?

Most lyophilized 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 (preferably −80°C), away from strong light, and under dry conditions.

A peptide solution once prepared should be used as soon as possible. If 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.

The shelf life of peptide solutions is limited, especially for peptides containing cysteine (C), methionine (M), tryptophan (W), asparagines (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 stability becomes worse when in a solution, especially at the higher pH (pH>8). We therefore recommend keeping solutions in the range of pH 4-6. It is recommended that peptides containing methionine, cysteine, or tryptophan residues be stored in oxygen-free atmosphere to avoid oxidation. The presence of dithiothreitol (DTT) can be useful in preventing oxidation.