

Guidelines for Peptide Dissolving

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Online Inquiry

What is peptide solubility?

Peptide solubility refers to the degree of solubility of a peptide in solution. Solubility is an important physical and chemical property that has an important impact on the solubility and stability of peptides. Although many peptides have good solubility in aqueous solutions, some peptides have low solubility and insolubility. When a peptide has a long hydrophobic amino acid sequence, this will pose a certain challenge to its dissolution. If you have relevant needs, we can provide you with a complete and tailor-made report on peptide solubility characteristics.

Factors affecting peptide solubility

The solubility of peptides is affected by many factors, including the nature of the solvent, temperature, pH, ionic strength and the structure of the peptide itself.

Solvent properties: Different solvents have different interactions with peptides. For example, DMS and DMF are suitable for dissolving hydrophobic peptides, while water or acidic buffers are suitable for dissolving acidic peptides.



Temperature: Increasing temperature usually increases the solubility of the peptide because high temperatures increase the molecular dynamic energy of the solvent and promote the interaction of the peptide with the solvent.

pH value: The acid–base properties of the peptide will affect its interaction with the solvent, and the solubility of the peptide will change at different pH values.

Ionic strength: High ionic strength may increase the interaction between the peptide and the solvent and reduce the solubility of the peptide.

Peptide structure: The sequence and structure of a peptide directly affect its interaction with solvent, thereby affecting solubility.

Methods to improve peptide solubility

Optimize amino acid sequence: Introduce hydrophilic amino acids to improve the hydrophilicity of peptides.

Adjust pH value: Select appropriate pH conditions, such as peptides are more soluble at pH other than isoelectric point.

Use of dissolution aids: Adding organic solvents such as DMSO and ethanol may promote dissolution.

Complex formation: Form soluble complexes with cyclodextrin, etc.

Increase the temperature: Heating the solution (< 40 °C) may help dissolve the peptide.

Ultrasonic treatment: Ultrasound helps stir the solution and improve dissolution.

Centrifugation: Always centrifuge the peptide solution before use to precipitate any undissolved peptide residue.

Peptide modification: For acidic peptides, we recommend [C-terminal acetylation](#) and for basic peptides, we recommend [N-terminal amination](#). Replacing the hydrophobic amino acids in the sequence with soluble amino acids can increase the solubility. Generally, Gly and Ala are used to replace the hydrophobic amino acid, and a better dissolution effect is generally achieved. PEGylation or the addition of D-amino acids to disrupt peptide chain aggregation can also increase solubility.

How to dissolve peptides?



Compared with ordinary biochemical reagents, peptides are more expensive and have unstable solution states, so researchers need to be cautious when using them. The dissolution of peptides is a very important step. Improper solvent selection may lead to insolubility or partial dissolution of the peptides, and even the experiment cannot be carried out. When selecting a solvent to dissolve peptides, solubility tests are usually carried out first with sterile water (especially oligopeptides

composed of only a few amino acids). For other special peptides, attention should be paid to the amino acid properties of the peptide, and then determine whether the peptide to be dissolved is acidic, neutral or basic.

Acidic Property	Amino Acids	Characteristics
Acidic amino acids	Glutamic acid (Glu), Aspartic acid (Asp)	Molecules contain one amino group and two carboxyl groups
Neutral amino acids	Glycine (Gly), Alanine (Ala), Leucine (Leu), Isoleucine (Ile), Valine (Val), Cysteine (Cys), Methionine (Met), Threonine (Thr), Serine (Ser), Phenylalanine (Phe), Tyrosine (Tyr), Tryptophan (Trp), Proline (Pro), Asparagine (Asn), Glutamine (Gln)	Molecules contain one amino group and one carboxyl group
Basic amino acids	Lysine (Lys), Arginine (Arg), Histidine (His)	Molecules contain two amino groups and one carboxyl group

General guidelines for peptide dissolution

Peptides with fewer than five residues are usually soluble in water or aqueous buffers unless the entire sequence consists of hydrophobic amino acids.

For peptides that are prone to aggregation, add 6M guanidine hydrochloride or 8M urea, followed by necessary dilutions.

Hydrophilic peptides containing > 25% charged residues and 25% hydrophobic amino acids are usually soluble in water or aqueous buffers.

Peptides containing 50% or more hydrophobic residues may be insoluble or only partially soluble in aqueous solutions.

Peptide solubility testing

Take a small portion for peptide dissolution test before peptide dissolution. You need to test several different solvents until you find the most appropriate one. Sonication helps break up particles and increase solubility. (Note: Sonication can cause solution heating and peptide degradation.)

Judging the acidity or basicity of peptides

Each acidic amino acid and the carboxy-COOH at the C-terminal in the peptide sequence is assigned

to +1, each basic amino acid and the amino-NH₂ at the N-terminal are assigned to -1, and the neutral amino acid is assigned to 0. Based on this, the value of the entire peptide is calculated. The final score is positive is called an acidic peptide, the score is negative is called a basic peptide, and the score is zero is called a neutral peptide.

Dissolution of acidic peptides

Try to dissolve with water first. If it cannot be dissolved with water, try to dissolve with 13% ammonia (v/v), and dilute the peptide solution to the required concentration. If the peptide sequence contains cysteine (Cys), it cannot be dissolved using alkaline solutions (in alkaline solutions, the cysteine molecule accepts protons and forms positively charged ions, which reduces solubility). Dimethylformamide (DMF) or N-methylpyrrolidone (NMP) can be used for dissolution.

Dissolution of neutral peptides

Usually dissolve with the smallest amount of strong organic solvent first. Try dissolving it first with methanol, isopropanol or acetonitrile. For highly hydrophobic peptides, first dissolve them with a small amount of DMSO (no Cys, Met, Trp in the sequence), and then dilute them to the required concentration. The organic solvent that dissolves the peptide is slowly added drop by drop to the aqueous solution. If the solution becomes turbid, it may have reached the dissolution limit and can no longer be dissolved.

Dissolution of basic peptides

Try to dissolve them with water first. If you cannot dissolve them, try to use 10%–30% acetic acid to dissolve them. If the peptide is still insoluble, it is recommended to try TFA(<50 µL) or pure acetic acid to dissolve it, and finally dilute the peptide solution to the required concentration.

Dissolution of easily aggregated peptides

For peptides with high Arg, Asp, Asn, Glu, Gln, His, Lys, Ser, Thr, and Tyr content (>75%) that are easy to aggregate (excessive intermolecular hydrogen bonds are easy to form in aqueous solution), you can use 6M guanidine hydrochloride or 8M urea to dissolve and prepare a storage solution, and finally dilute to the required concentration.

Recommendation: In order to prevent or minimize the degradation of peptides, please store the peptides in the form of lyophilized powder at -20 °C, preferably -80 °C. If it is necessary to store solution peptides, it is best to store them in small samples to avoid repeated freezing and thawing. If a sample is not used up after thawing, it should be thrown away. Bacterial degradation can sometimes become a problem for solution peptides, so please dissolve the peptides in sterile water or filter the peptide solution to sterilize.

