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- Substrates & Inhibitors
- dPEGs and Linkers

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Key literature citations and patents referring to Peptides International products and services

EXCELLENCE COMPOUNDED

- High Purity, Quality and Value
- Strict Confidentiality
- Friendly Service
- Proactive Project Management

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PEPTIDES INTERNATIONAL

Custom Services Portfolio

Custom Peptide Guidelines

Peptide Storage and Shipping

How stable is a peptide?

Most peptides experience very little degradation over time and are stable for more than 1 year if they are stored in a lyophilized state at -20 °C or below and protected from moisture and light. However, following reconstitution of a peptide in solution, stability and storage time will decrease. We do not test the stability of a peptide solution; therefore, prompt use in recommended. However, if you decide to store a peptide solution for several weeks, you should aliquot out the solution into clean, inert glass or plastic vials to prevent freeze-thaw cycles.

Conditions that can affect peptide stability include the following:

- Contamination from microorganisms or metal ions can lead to peptide-bond cleavage. Use sterile buffer or water to reconstitute the peptide.
- Moisture can lead to hydrolysis of the peptide. The peptide should be allowed to warm gradually to room temperature in a desiccator to reduce condensation of water vapor.
- Constant freezing and thawing can compromise peptide integrity; therefore, stock solutions should be aliquoted
- Peptides containing Cys or Met are susceptible to oxidation due to the side chain groups with oxygen. It is advisable to blanket the peptide with argon or nitrogen when the vial is opened. Buffers used to dissolve these peptides should be degassed, either by bubbling argon or nitrogen through the solution for 10 minutes, or by subjecting the solution to high vacuum for 10 minutes using a common ultrafiltration capsule. Peptides containing such amino acids tend to have very short-term stability, and long-term storage is not recommended. In some cases, peptides containing Trp may be hygroscopic and require similar handling methods.
- Certain amino acid bonds in a peptide are more problematic:
  - Asp-Pro bonds are sensitive to acid cleavage.
  - Asn-Gly and Asp-Gly bonds, and sometimes Asp N-term to short side residues (Ser, Thr, Ala, Asn), can cyclize to form an aspartimide intermediate which, in turn, can undergo spontaneous changes that can alter the peptide.

What are conditions for shipping for a custom peptide?

Custom peptides are shipped at room temperature and delivered in a lyophilized state. These shipping conditions do not compromise quality since these products are stable at room temperatures and above for as long as several weeks. Custom peptides containing Cys or Met are susceptible to oxidation due to the side chain groups with oxygen. These peptides are packaged under argon gas in order to displace oxygen and reduce likelihood of oxidation. In some cases, peptides containing Trp may be hygroscopic and require packaging under argon gas as well.

How should a peptide be stored?

Once a product arrives at your facilities, it should be stored at -20 °C or below. The peptide should be stored with a desiccant to maintain a dry environment.

How should a peptide solution be prepared?

Since there is not a universal solvent for dissolving every peptide, this crucial step is not always straightforward as it may appear. Because several solvent systems may be necessary until the desired conditions are achieved, always test a small sample of the peptide to determine the best solvents for complete solubilization. When testing, begin with solvents that can be easily removed by lyophilization, such as water and acetic acid. For this reason, it is not recommended to start with buffers which have high salts concentrations.

Determine Overall Charge

Charged amino acids aid solubility in aqueous environments. A sequence with little or no overall charge at any pH is not likely to be water soluble.

- Hydrophobic amino acids: Ala, Phe, Ile, Leu, Val, Pro, Met, Trp, Tyr, Cys
- Positive Charges: Lys, Arg, His and Free N-terminus
- Negative Charges: Asp and Glu and Free C-terminus

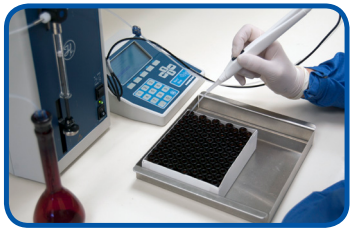
First determine if the peptide is charged or neutral by calculating the overall charge of the peptide at pH 7. Calculate overall charge by using the following values:

- $^{+}$1 for each basic residue (Lys, Arg, and N-terminus)
- $^{-}$1 for each acidic residue (Asp, Glu and C-terminus)
- For His, use $^{+}$1 at pH <6 and zero at pH >6

If the overall charge of the peptides is negative then the peptide is acidic, if it is positive then it is basic, and if zero then it is neutral.

Charged Peptides

For acidic peptides (and/or if the total number of charges of the peptide at pH 7 is greater than 25% of the total number of residues): use a small amount of 0.1M ammonium bicarbonate to dissolve the peptide, and then dilute it with water to the desired concentration. Maintain pH around 7 and adjust pH as needed.



For basic peptides (and/or if the total number of charges of the peptide at pH 7 is between 10-25% of the total number of residues): use a small amount of 25% acetic acid to dissolve the peptide and dilute it with water to the desired concentration. Adjust pH with 8 M NH_4OH to desired pH for oxidation (4-7).

For neutral peptides (and/or if the number of charges is greater than 25% of the total number of residues): use the strategy described for acidic peptides. Otherwise, the use of organic solvents is recommended.

Hydrophobic or Neutral Peptides

Hydrophobic peptides containing 50% to 75% hydrophobic residues may be insoluble or only partially soluble in aqueous solutions, even if the sequence contains 25% charged residues. It is best to first dissolve these peptides in a minimal amount of stronger solvents such as acetonitrile, isopropyl alcohol, ethanol, and/or acetic acid, and then slowly add (drop wise) the solution to a stirred aqueous buffer solution. If the resulting peptide solution begins to show turbidity, you might have reached the solubility limit and it will be futile to proceed. Again, it is important to remember that the initial solvent of choice should be compatible with the experiment.

It is important to dissolve the peptide completely in the initial solvent (such as acetic acid, acetonitrile) because the rate of dissolution of peptides into these solvents is usually higher than in a water/solvent mixture. If a water/solvent mixture is used first to dissolve the peptide, the final volume of solution may be larger than necessary.

Sonication

It may be necessary to sonicate the solution before determining if the solvent choice was appropriate. Sonication should improve solubilization by breaking the solid peptide into smaller particles. If the solution gels, becomes cloudy or turbid, or has visible particulates, the peptide has not dissolved completely but is suspended. At this point, a stronger solvent is necessary and the solvents should be removed. Begin again with the dry sample.