

Peptide Storage

For the most effective long-term storage of peptides it is advised to store as a lyophilised powder at -20 C. Repeated cycles of freeze-thaw should be avoided. If only small quantities are required at a time then aliquot before freezing. Peptide Synthetics can aliquot your peptide into smaller quantities for you on request, e.g. 10mg split into 1mg aliquots.

Exposure to moisture will greatly decrease long-term stability of lyophilised peptides. To prepare the peptide for use, remove from cold storage and allow the peptide to equilibrate to room temperature before removing the lid of the container.

If your peptide requires special treatment you will be informed when the peptide is sent to you.

General Guide for Dissolving Peptides

- The more charged residues on a peptide, the more soluble it is in aqueous solutions.
- First try to dissolve a small amount of the peptide in either water or buffer.
- Consider that any solvent that you use must be compatible with your assay.
- Gentle warming and sonication are effective tools in aiding peptide solubilisation. Be aware that a peptide may appear to have solubilised after sonication, but if the solution is cloudy or has gelled it may merely be in suspension.
- If a peptide does not dissolve and you wish to recover it, lyophilise to remove your solvent.

There is no general solvent for suitable for solubilising all peptides; the flowchart below provides a general **guideline** for peptide solubilisation. Please select the best solvent for your supplied peptide by testing a small amount first. It is advisable to first attempt to use solvents that are readily removed by lyophilisation.

The solubility characteristics of a peptide can be gauged by the amino acid composition; the ionic charges on the peptide will help towards determining its solubility. Please note that although hydrophilicity analysis will assist in the ability to solubilise your product, it is not a definitive determinant of the solubility of the peptide.

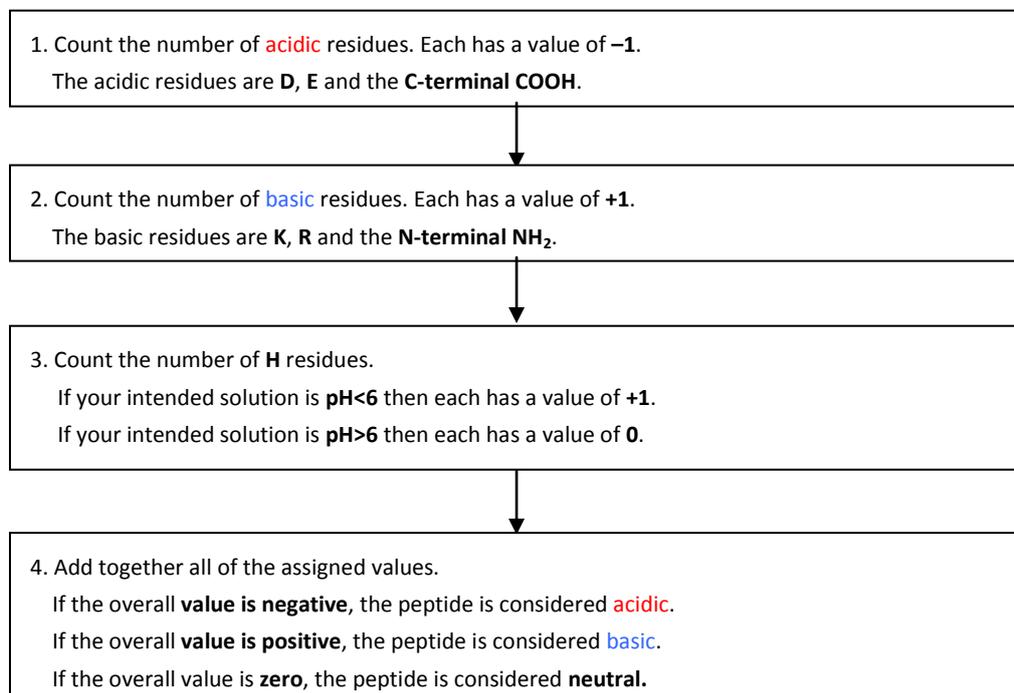
Peptides containing free cysteines should be dissolved in degassed acidic buffers. The thiols will be oxidized at pH>7 forming disulphide bonds. Solutions should be prepared immediately before use to avoid air oxidation.

Peptides containing a large percentage of hydrophobic residues, along with the peptides already described in the "Approaches to Peptide Solubilisation" flowchart, require the use of organic solvents due to their largely uncharged nature. Recommended solvents are DMSO, DMF, acetic acid, acetonitrile, methanol, propanol or isopropanol.

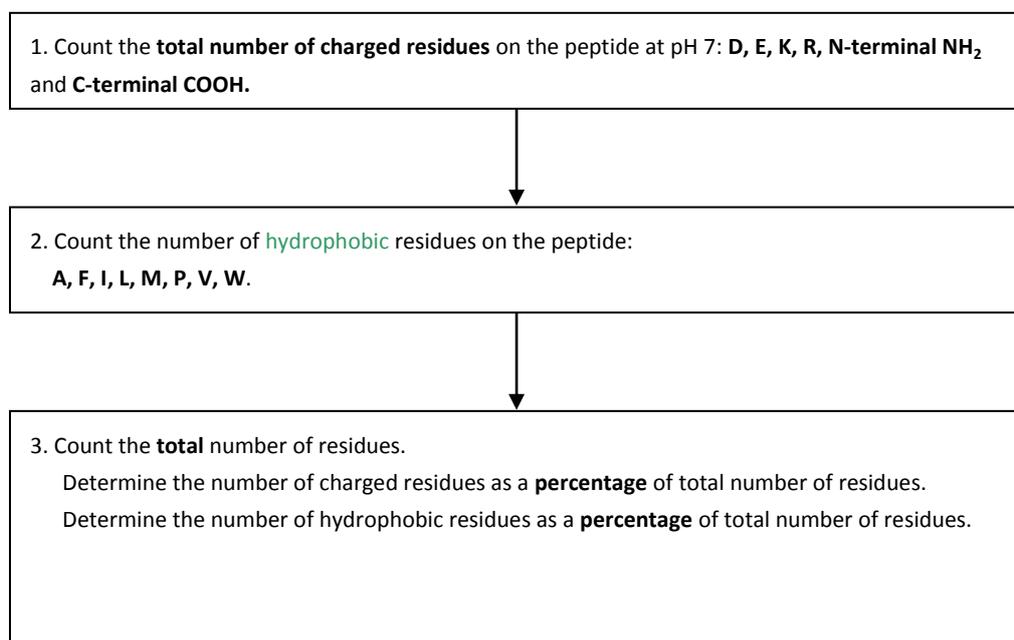
The peptide should be dissolved in the organic solvent completely before dilution in water with aqueous buffers (Sonication may be required). The buffer solution should be added dropwise with gentle agitation throughout the addition. More organic solvent may be added if the peptide starts to precipitate.

DMSO is a good solvent and has the advantage of being tolerated by cells. It is however, difficult to remove by drying. DMSO should not be used if the peptides contain methionine (M) or free cysteine (C). Its use may cause oxidation of the side-chain functionalities if left in solution for long periods.

Calculating the Overall Peptide Charge



Calculating the Percentage of Charged & Hydrophobic Residues



Hydrophilicity Analysis – Example 1

MART-1 Peptide

ELAGIGLTV

For the above peptide we can carry out a hydrophilicity analysis as an example and deduce the appropriate solvent.

One **acidic** residue and one C-terminal COOH = -2

No **basic** residues and one N-terminal NH₂ = +1

Overall charge = -1

The peptide is considered **acidic**.

Percentage charged residues = $(1/9) \times 100$
= 11 %

This is very close to the 10% limit for using organic solvent (see “Approaches to peptide solubilisation” flowchart below). Also, almost half of the residues are **hydrophobic**, so it is best to initially use an organic solvent for this peptide. Dissolving the peptide in the minimum amount of DMSO and then slowly adding dropwise to the desired aqueous buffer solution whilst constantly agitating would be an appropriate method.

Hydrophilicity Analysis – Example 2

OVA (323-329)

ISQAVHAAHAEINEAGR

Two **acidic** residues and one C-terminal COOH = -3

One **basic** residue (assuming neutral pH and ignoring the two H residues) and one N-terminal NH₂ = +2

Overall charge = -1

Peptide is considered acidic at neutral pH.

Percentage charged residues = $(3/17) \times 100$
= 18 %

Percentage Hydrophobic residues = $(3/17) \times 100$
= 18 %

This peptide has quite a low percentage of hydrophobic molecules, evenly spaced throughout the peptide. It will be soluble in water, but if it did not dissolve and were applied to the flow diagram for the dissolving approach, the output would be:

Add a small amount of 10-25% acetic acid and dilute with water to the desired concentration.

