

## Peptide Handling Guideline

Version 4.2, Revision 2011-09-08

### Reconstitution Guideline

Proper peptide handling and solubilization is the starting point of a successful bioassay project, and we believe this handling guideline will help you dissolve your peptides properly. On CoA along with each peptide delivery, you may also see reconstitution conditions which we have used in the peptide purification process – this is for your reference only, you may dissolve your peptide in a different solvent according to your assay needs.

- ◆ Use only a small aliquot of peptide to test the dissolution method. Once satisfied, apply to the larger aliquot as needed.
- ◆ In principle, solvent used should be the solvent that will facilitate or be compatible with your experiment. However, we shall also keep in mind that there might be a challenge sometimes to find an “ideal” solvent which will solubilize peptides, maintain their integrity and be compatible with biological assays.
- ◆ For initial solvent used should be the most appropriate one. For example, for a very hydrophobic peptide, it is better to dissolve it in a small volume of organic solvent (such as DMSO or acetonitrile) before applying the aqueous solution. In other words, adding organic solvent to a suspension of hydrophobic peptide in aqueous solution is not likely to help much in dissolving.
- ◆ Peptide solution might be unstable at temperatures even lower than -20°C. As such, a peptide solution once prepared should be used as soon as possible.

### What solvent(s) I can use to dissolve my peptides?

If it is a short peptide which is 5aa or less, try sterile distilled water first and it is likely to dissolve.

For other peptides, the overall charge of the peptide will help determine which initial solvent to use. Assign a value of -1 to acidic residues which include Asp(D), Glu(E), and the C-terminal free acid(-COOH). Assign a value of +1 to basic residues which include Arg (R), Lys (K), His (H), and the N-terminal free amine(-NH<sub>2</sub>). Calculate the overall charge of the entire peptide.

1. If the overall charge of the peptide is positive (a basic peptide), try to dissolve the peptide in sterile distilled water first. If water fails, add ~20% acetic acid solution. If the peptide still does not dissolve, add drops of TFA (< 50ul), or use 0.1%TFA/H<sub>2</sub>O to solubilize the peptide. Then dilute the peptide solution to the desired concentration.
2. If the overall charge of the peptide is negative (an acidic peptide), try to dissolve the peptide in sterile distilled water first. If the peptide persists as visible particles, sonication can be tried. If water fails, add NH<sub>4</sub>OH (<50ul) or 0.1%NH<sub>4</sub>OH drop-wise. Then dilute the peptide solution to the desired concentration. If the peptide contains Cys, do NOT use basic solutions (NH<sub>4</sub>OH), but use DMF instead.
3. Peptide whose overall charge is zero (the peptide is considered neutral). It usually dissolves in organic solvents, such as acetonitrile, methanol, or isopropanol. If this does not dissolve completely:
  - a) For peptides that tend to aggregate (due to the hydrophobic interaction), the addition of denaturants, such as 8M urea or 6M guanidine-HCl, may also be required.
  - b) For very hydrophobic peptides (containing more than 75% hydrophobic residues), add DMSO drop-wise (use DMF instead for Cys containing peptides), and then dilute the solution with water to the desired concentration.

### Storage Guideline

Most lyophilized peptides shall be stable at room temperature for at least a few weeks. For long term storage, it is strongly recommended that you store peptide in powder form at -20°C or lower, away from strong light, and under dry condition. 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), asparagine(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. Avoid DMSO if the peptide contains Met, Cys or Trp, due to sulfoxide or disulfide formation. 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.

Since peptides in solution are unstable at temperatures even lower than -20°C, a peptide solution once prepared should be used as soon as possible. If storage in solution is unavoidable, use sterile buffers at pH 4-6 and store in aliquots at -20°C to prolong the storage life.