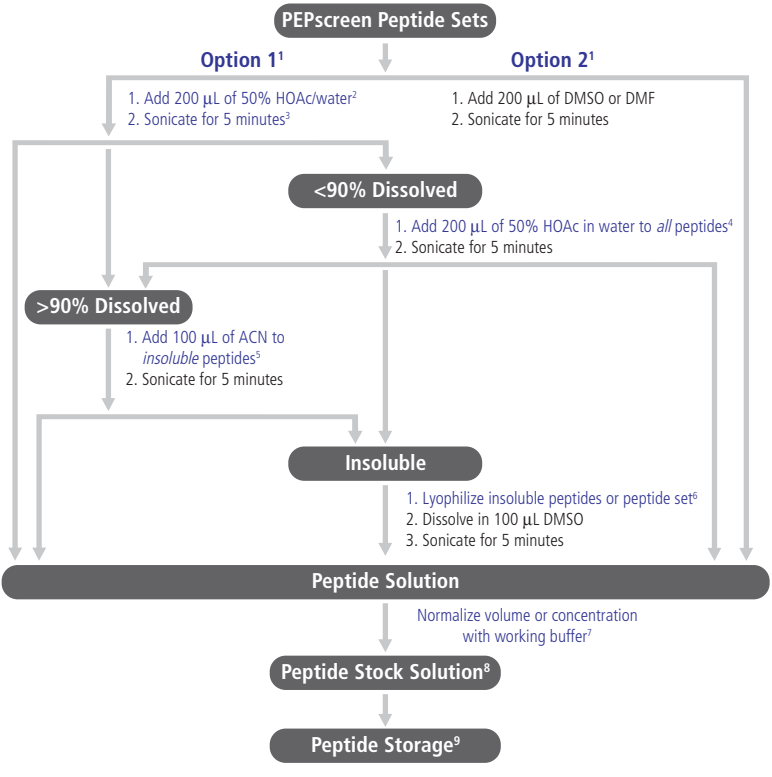
# Guidelines for Dissolving PEPscreen® Peptide Sets

Complete solubilization of peptides is important for successful screening of peptide activities. Peptides can be fully active only if they are completely solubilized and are able to assume the correct conformation for binding to their receptors. As the number of peptides in a set increases, so does the potential solubility variation of the peptides within the set. Therefore, in order to obtain accurate and reliable peptide activity data, careful attention should be devoted to the process of dissolving peptide sets.

The strategy for dissolving the PEPscreen peptide set is different from dissolving individual peptides. For individual peptides, conditions are chosen for optimum solubility based on the given peptide sequence. However, for peptide sets, conditions are chosen in an effort to dissolve as many of the peptides in the set as possible in the first solubilization attempt. Suggested common strategies are schematically represented below:

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## Notes

1. The choice of options to follow is dependent on the specific *application* of the peptide sets. If the peptide application cannot tolerate dimethylsulfoxide (DMSO) or dimethylformamide (DMF), Option 1 is recommended. A dilute solution of acetic acid (HOAc) and/or acetonitrile (ACN) is less toxic to cells and can be easily removed by lyophilization, if necessary. In addition, the slightly acidic condition maintains the stability of the peptide against oxidation. However, not all of the peptides within the set may dissolve.

   When DMSO or DMF is tolerated in the peptide application, Option 2 is suggested because it is very likely that all of the peptides will dissolve. While DMSO is generally considered the better solvent, DMSO is a mild oxidant and may oxidize the Cys, Met, and Trp residues in peptides. Therefore, DMF is a better choice if the sequences contain many of these sensitive residues. Unfortunately, both DMSO and DMF are very difficult to remove by lyophilization or by other means and, therefore, any solvent exchange, if necessary, is difficult.

2. The amount of HOAc can be varied according to the peptide application. The concentration and volumes suggested here are for optimal solubilization. However, it is important to ensure that the amount of HOAc added does not affect the pH of the final assay solution. A rough test would be to mix the calculated amount of HOAc in the aliquot of the peptide solution with the application buffer and measure the pH of the resulting solution. Other variables such as volume of the peptide aliquot, concentration of buffer, volume of final assay solution, etc., must be carefully pre-determined to ensure the integrity of the assay.

   Peptides in aqueous solutions containing high concentrations of acetic acid can become hydrolyzed at room temperature. These solutions should be diluted with buffer within a day after solubilization to bring the acetic acid concentration to less than 10%.

3. Sonication for longer periods of time may help in the solubilization process. However, sonication for over 30 minutes *in this step* is not recommended because of potential hydrolysis of the peptides in the 50% acetic acid solution.

4. Since this repeat step will increase the concentration of acetic acid in solution, its compatibility with the peptide application should be ascertained. If high acetic acid concentration is a potential problem, the following options may be explored according to preferences: (1) adding 200 µL 50% ACN (acetonitrile) into the peptide *set*, (2) lyophilizing the peptide *set* and redissolving in DMSO or DMF, or (3) treating only the *insoluble* peptides with 50% acetic acid.

5. Where the particular peptide application allows, it is better to add 50% ACN into *all* of the peptides, instead of only to the *insoluble* peptides, to ensure complete solubilization, as well as to have uniformity of all peptides in the sample set.

6. Again, depending on the application and the relative number of the insoluble peptides, one may exercise the option of lyophilizing and redissolving in DMSO or DMF the whole peptide *set* or only the *insoluble* peptides.

7. Ideally, one would normalize the final peptide concentration. However, there are situations where normalizing the final volume of the stock solution might be more practical. This includes situations where some of the peptides in a set are dissolved in different ways, or there are no quick and practical ways to determine the peptide concentrations *before* dilution of the peptide set. The most practical approach is to normalize the final volume, use uniform volumes of aliquots for the assay, and finally normalize the *data* based on the peptide concentrations in the stock solutions.

8. Whenever possible, it is recommended that the concentration of the stock solution be around 1-2 mg of peptide per mL of solution. This is dilute enough to minimize the potential precipitation of the peptides during storage, but concentrated enough to take relatively small volumes (<100 µL) of aliquots for the assay, and therefore minimizing the effect of the solvents initially used for solubilization.

9. Peptide stock solutions can be stored at 4°C for short-term storage (less than 1 week), frozen at –20 °C for medium-term storage (less than 1 year), or at –80 °C for long-term storage (more than 1 year). Depending on the volume and frequency of use, it might be more practical to aliquot the stock solutions into suitable amounts that will minimize freeze-thaw cycles.

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