Guidelines for Handling Cleaved PepSets™

Peptide libraries (PepSets) from Mimotopes are supplied in either of two formats: "Non-cleavable", where the peptides remain permanently attached to the solid phase on which they were synthesized; and "Cleaved", where peptides are supplied as a powder in labelled tubes.

The first step in using a Cleaved PepSet is to dissolve the peptides to obtain a homogeneous solution which can be aliquotted, diluted, assayed and stored. Dissolving the peptides is thus a critical step in the successful use of a PepSet. Here, we offer our advice on choices of solvents, and a methodology for you to achieve the best results in carrying out this step.

Due to the large number of peptides in a PepSet it is generally not practical to vary the handling method for each peptide. Most users opt to apply a single method to all peptides. The methods discussed below have been prepared with this approach in mind.

Topics in this article:

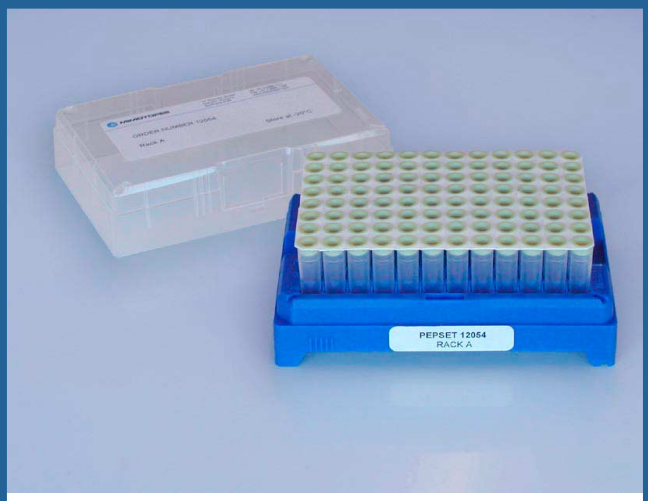

Figure 1. A PepSets peptide library ready for use

Aspects of Peptide Solubilization

There is no "universal" solvent which is suitable for dissolving all peptides. The amino acid composition and sequence of each peptide determine its solubility in a given solvent. For example, peptides with a majority of charged residues, such as K(Lys), R(Arg), H(His), D(Asp) and E(Glu), will generally be more soluble in aqueous solvents, whereas those with a greater number of hydrophobic residues, such as A(Ala), V(Val), L(Leu) and F(Phe) will generally be more soluble in organic solvents, such as dimethyl sulfoxide (DMSO) or dimethylformamide (DMF).

All peptides will have some degree of solubility in the solvent being used, and depending on the application, the amount of peptide in solution may be sufficient to obtain valid screening results, even for peptides exhibiting low solubility.

Choice of Solvent

Factors influencing choice of solvent include:

1. Effectiveness of the solvent in dissolving a wide range of peptides
2. Desirable properties of the solvent such as: freedom from toxicity, low chemical reactivity, high stability against decomposition, low volatility (low loss by evaporation)
3. Availability of the solvent in pure form at reasonable cost
4. Ease of handling

Clearly, pure water would be the "first choice" under many of these criteria, but where some of the peptides are hydrophobic and unlikely to dissolve in water, other solvents or solvent mixtures need to be considered. Many users preferentially use DMSO or DMF, which are widely acceptable in many end use applications for peptides, and have the ability to dissolve most peptides.

We recommend using 0.2mL of a 80%(v/v) DMSO/ 20%(v/v) water mixture as the most generally useful solvent for cleaved PepSets, for example, when the peptides are for use in ELISA tests. However, for applications where the mild toxicity of DMSO and DMF for living cells is not acceptable, or where these solvents are not considered inert enough and any effect on the properties of the peptides is unacceptable, other solvents need to be chosen. Further discussion about DMSO and other solvents can be found on the back page of this Note.

The following descriptions relate to just one preferred solvent mixture (acetonitrile/water), and while the handling protocols are fairly "generic", they may need to be modified if other solvents are being used.

Many peptides are soluble in a 50% (v/v) acetonitrile/water mixture, which is thus a good general solvent for PepSets. This solvent mixture also has the benefit that if an individual peptide in the PepSet is not readily soluble, then the solvent can be completely removed by freeze drying so that another solvent can be tried. Acetonitrile is a widely used solvent for reverse phase HPLC and is remarkably nontoxic for cultured cells, provided a high purity grade is used. A high concentration of acetonitrile (50% acetonitrile in water) is recommended to dissolve the peptides, and most applications will require a further dilution in aqueous buffers, to a degree where solvent toxicity is no longer a consideration. For example, many cells will grow in tissue culture medium containing 1% (v/v) acetonitrile, although we do not recommend using such high levels if it can be avoided. A final acetonitrile level of 0.3% (v/v) or less should be safe for all common cell culture systems.

Warning: always take appropriate precautions against personal exposure to all solvents.

Dissolving the Peptides

The following is a practical procedure based on our experience of dissolving PepSets. Your PepSet may contain traces of the antioxidant propanedithiol when you receive it, which will be evident as a thiol smell upon opening the PepSet container. This is not a cause for concern because the amount of residual propanedithiol is vanishingly small and is only detectable because of its high pungency.

1. Wearing clean, solvent resistant gloves, remove the strip caps from all the tubes (Figure 2). The use of a decapper tool as shown in the photograph is recommended. A decapper is shipped with each cleaved PepSet. Using an 8 tip Multichannel pipetter add 400µL (or 2×200 µL) of 50% (v/v) acetonitrile/water solvent to each tube.

2. Recap the tubes with the strip caps making sure that each tube is sealed correctly. Incomplete capping could result in solvent leaking from the tubes. Throughout all the cap removal and replacement operations it is important that the caps and tubes are not mixed, i.e., each cap should always only be placed back onto the tube it was originally on. If there is any doubt as to which caps belongs to which tubes, either thoroughly wash and dry all the caps before recapping, or use new caps. Cross-contamination of peptides should be strictly avoided as this may lead to invalid results.

3. With the tubes secured in the rack, such as by placing a gloved hand firmly across all lids or by replacing the rack lid, repeatedly invert the tubes for approximately 5 minutes to thoroughly mix the solvent and peptide, and enable dissolution to take place.

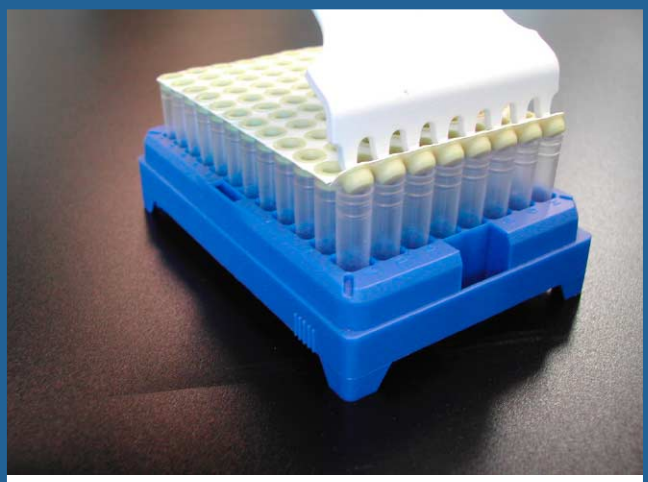

Figure 2. Decapping the tubes

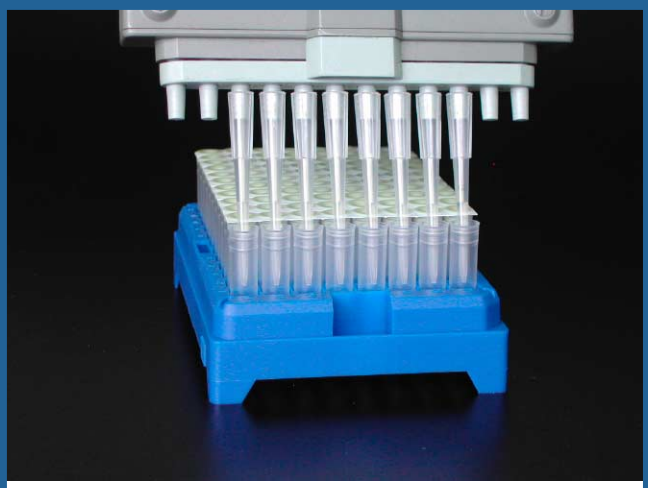

Figure 3. Adding the Solvent

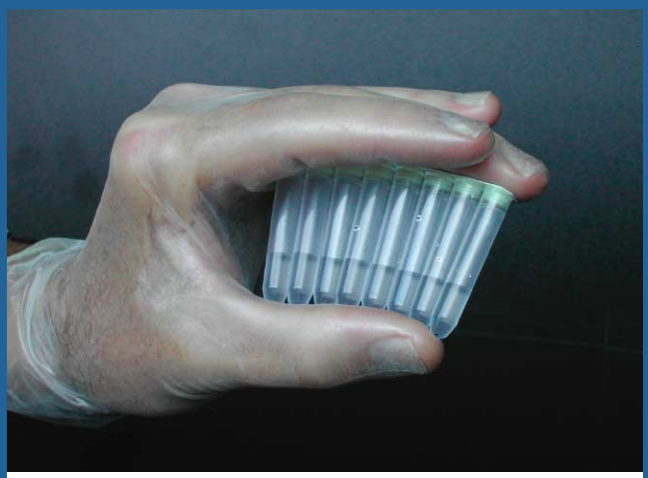

Figure 4. Visual Examination

4. Rows of 8 capped tubes can be removed from the rack for inspection, the top of the tubes being held by a finger and the bottom of the tubes being held by the thumb. Gentle inversion will then reveal whether or not the peptides have dissolved.

5. Repeat this inspection for each row, noting those peptides that may not be completely dissolved. If a sonication bath is available then the rows of tubes can be held in the sonicator bath for around 5 minutes to further aid dissolution. Ensure that the bottom of each tube is in direct contact with the water. Avoid contact of the water with the top of the tubes; avoid warm water which could pop the caps due to acetonitrile vapor pressure building up. If, after this procedure, some of the peptides are still not completely dissolved, then there are two general ways to proceed, as described below.

6. Where many peptides do not appear to be completely dissolved, then a first approach is to add additional aliquots of the acetonitrile/water mixture, and repeat the mixing and sonication process. A total volume of up to 0.7mL is practical for use in the tubes as supplied. Use of a larger amount of solvent would require transfer of each peptide to a larger tube. The peptides which are still undissolved at this point can alternatively be dried down and another solvent can be tried, as in the following procedure.

7. If there are only a few peptides in the whole PepSet that appear to be insoluble, pre-label a 10mL polypropylene tube corresponding to each undissolved peptide. One at a time, remove the cap from the corresponding tube of undissolved peptide (caps can be separated from each other with small scissors or a scalpel) and transfer the contents of the tube into the pre-labeled 10mL polypropylene tube. Freeze-dry the 10mL tubes to remove the solvent. An examination of the sequence of the peptide to see which amino acid residues are present should provide some clues as to what solvents may now be used in a second attempt to dissolve each peptide. DMSO is probably the most suitable solvent for hydrophobic peptides, but see below for a discussion of other possible solvents, and the disadvantages of DMSO. When the peptides in the 10mL tubes are dry, add the same volume of the second solvent (e.g. DMSO) as that originally added to all peptides (400µL), so as to maintain an equivalent peptide concentration for all of the peptides. If the peptide does not completely dissolve, then mix thoroughly on a vortex mixer. If available, use a fine tipped sonic probe at low power to disperse the peptide in the DMSO, which will aid dissolution. Even if the peptide does not dissolve completely, dispersing the peptide into a fine suspension will assist subsequent sampling because there is a greater likelihood that a uniform amount of peptide will be obtained each time it is sampled. Carefully transfer the peptide solution or suspension back into its original tube, cap the tube, and position the tube in its original location in the PepSet rack.

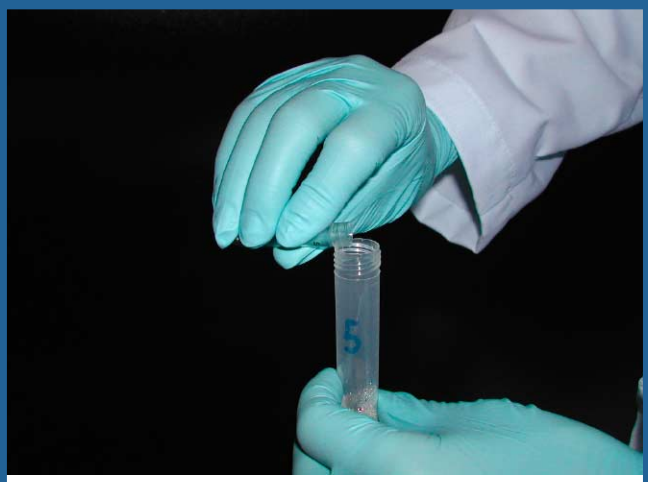

Figure 5. Transfering to a 10mL tube

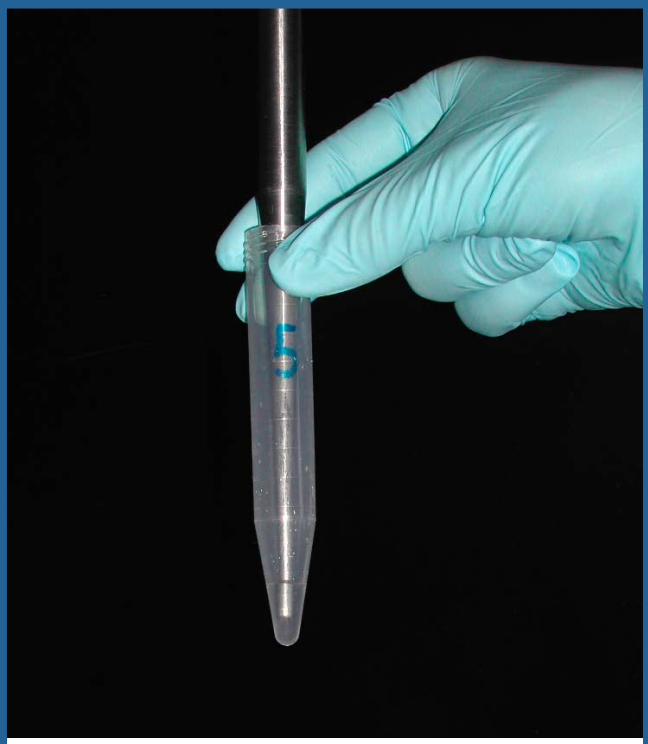

Figure 6. Using a sonic probe

It is important to take a pragmatic approach when using PepSets. Even though some peptides may not appear to be completely dissolved, there will almost certainly be enough dissolved peptide to yield meaningful results in many applications, such as binding assays where an excess of peptide is normally present.

Handling the Peptides after Dissolution

This now is the master (parent) set of stock peptides and it should be emphasized that whenever the solutions are sampled great care should be taken not to cross-contaminate the peptides.

Depending on the intended use of the PepSet it might be useful to dispense aliquots of these master solutions into polypropylene microtitre plates or new sets of capped polypropylene tubes, to make replicate sets.

For most applications it will be necessary to further dilute the parent PepSet. Whenever this is done, care should be taken not to allow cross-contamination to take place. Simple procedures like only uncapping one row of tubes at any one time can greatly reduce the possibility of cross-contamination. The parent and replicate sets can be stored frozen at -20°C or colder until required for use.

Dissolved peptides should not be held for long periods of time in the liquid state. The peptides may degrade by chemical processes, or even worse, growth of microbiological contaminants may lead to appearance of new proteins/peptides while the original peptides are degraded.

If storage in the liquid state is unavoidable, the solution should be one in which microbiological growth will not occur, such as a solution with a high level of organic solvent or an added inhibitor such as 0.1%(w/v) sodium azide.

Quality of Solvent

The highest grades of solvent should be used for dissolving your PepSet. For example, water should be high purity, essentially free of salts, oxygen and pyrogens. Freshly distilled or reverse osmosis (high resistivity) water from a well maintained system is generally suitable, but may need to be de-aerated prior to use if the oxidation of sensitive residues such as cysteine, methionine and tryptophan is to be avoided. Peptides are supplied in the reduced state, having been protected from oxidation during preparation by use of the protectant propanedithiol. When you receive the PepSet, the presence of traces of propanedithiol may be evident as a thiol smell upon opening the PepSet container.

For the other components of the solvent mixtures, we recommend HPLC grade solvents. Lower grades of solvent may contain toxic impurities or components which will cause false positive or negative results in your experiments. Even old bottles of high purity solvents may be unsuitable due to gradual degradation of solvent over time (e.g. DMF) or accidental contamination of the bottle contents by other users.

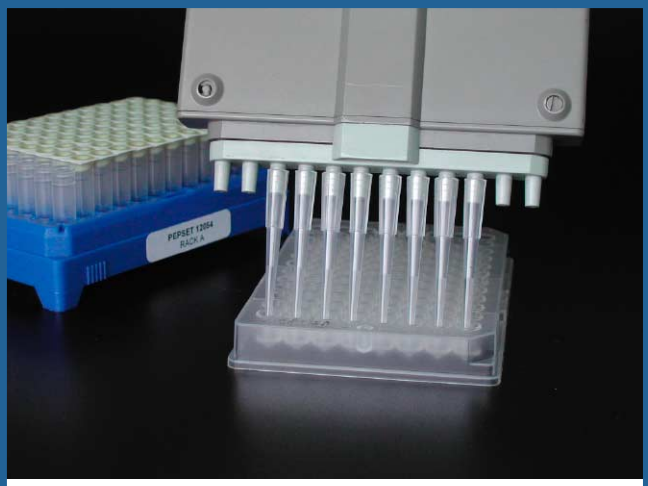

Figure 7. Aliquotting peptides

Other Choices of Solvent

Mimotopes' standard buffer for dissolving peptides before final freeze drying is 5% (v/v) acetic acid/40% (v/v) acetonitrile in water. The acetic acid concentration is sometimes increased to 20% if difficulty is experienced with dissolving some peptides, but if this is done, the peptides should be freeze dried (or frozen) quickly, to avoid side reactions.

If DMSO is used, it may help to add a little water to the DMSO (e.g. 20%) to assist hydration of highly hydrophilic peptides (e.g. Highly charged peptides). DMSO is a weak oxidizing agent and its use can result in gradual oxidation of cysteine-containing peptides to dimers or polymers through disulfide bridge formation. Peptides containing methionine or tryptophan can also be affected. To avoid this effect a different solvent such as DMF or its more stable cyclic analog, N-methylpyrrolidone (NMP) can be used.

Please refer to our other Literature on handling and storing peptides for more ideas on sequence-specific approaches to peptide dissolution, or contact the Peptide Support Group at: peptide_support_group@mimotopes.com

Mimotopes International
Suite 1, Level 1, Unit 9, 677
Springvale Rd, Mulgrave
VIC, Australia 3170
mimotopes@mimotopes.com
www.mimotopes.com

Mimotopes Asia Pacific
Tel: +61 3 9565 1111
Fax: +61 3 9565 1199
australia@mimotopes.com

Mimotopes Europe
Tel: +44 870 460 1500
Fax: +44 870 460 1501
europe@mimotopes.com

Mimotopes US East
Tel: +1 651 603 0909
useast@mimotopes.com

Mimotopes US West
Tel: +1 651 603 0909
uswest@mimotopes.com