

Storage and Handling of PEPscreen® Custom Peptide Libraries

The first step in working with your PEPscreen® peptide set is to obtain a uniform solution of dissolved peptides, which can be aliquotted, diluted, assayed, and stored. Dissolving the peptides is a critical step for successful use of a PEPscreen® peptide set. This document provides guidelines for dissolving, handling and storing your peptide set.

When working with a peptide library, it is most practical to follow a single solubilization strategy for the entire set of peptides.

Peptide Solubilization

Unfortunately, there is no universal solvent suitable for dissolving all peptide types that is compatible with all biological assays. The solubility of a particular peptide is dependent on the amino acid composition and the sequence. Sequences comprised of several charged residues (D, E, K, R, and H) are soluble in aqueous solvents. Sequences comprised of several hydrophobic residues (A, V, M, L, I, F, and W) are more soluble in organic solvents such as N,N dimethylformamide (DMF), dimethyl sulfoxide (DMSO), or N-1-methyl-2-pyrrolidone (NMP).

All peptides possess a certain level of solubility in a solvent. Depending on your application, it may not be necessary to dissolve the entire peptide sample to achieve valid results in your screening experiment. When choices are available for specific sequence characteristics, it is advisable to try solvents that are more easily removed by lyophilization, in the event the solvent does not dissolve the sample.

Choosing the Solvent

The following characteristics should be considered when choosing your solvent:

- The ability of the solvent to dissolve a variety of peptide types
- Solvent characteristics: toxicity, chemical reactivity, and stability
- Solvent purity
- Solvent availability and cost
- Special handling requirements

Pure water is often the preferred choice; however, there are likely hydrophobic peptides in your peptide library

that will be insoluble in water. The preferred solvent is either DMF or DMSO because of their ability to dissolve a wide variety of peptide sequences, and they are relatively benign on the peptides and the assays.

In general, we recommend a solution of 0.2 mL of 80% DMSO/20% water mixture (v/v) as the optimal solvent for peptide sets. However, in applications involving living cells, the final concentration of DMSO or DMF must be minimized by multiple-fold dilutions due to the mild toxicity of these solvents. If the tolerance of specific biological assays to DMF or DMSO is not well established, other suitable solvents will need to be explored.

Solubilization Guideline

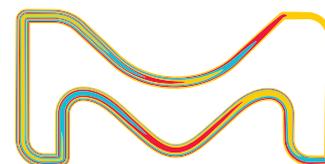
The guideline below is one example of a useful solvent mixture, acetonitrile (ACN) and water (H₂O).

A good solvent for dissolving peptide sets is a 50% (v/v) ACN/H₂O mixture because many peptides are soluble in this mixture. Another benefit of this solvent mixture is the solvent can be completely removed from the peptide sample by lyophilization if the peptide proves to be insoluble in the ACN/H₂O mixture. Once the sample is lyophilized, another solvent can be tried. Acetonitrile is a widely used solvent and is less toxic to living cells, provided a high-purity grade is used. It is recommended that a high concentration of acetonitrile (50% ACN in H₂O or greater) be used for dissolving the peptides. Many applications require dilution in aqueous buffers to remove solvent toxicity. A final acetonitrile level of 0.3% (v/v) or less should be safe for common cell culture systems.

Dissolving the PEPscreen® Peptide Set

The guideline below is a useful procedure for dissolving peptide sets.

1. Remove the caps from the tubes containing the peptides you wish to dissolve. It is advised that you uncap the tubes individually or in 8-column rows. A "decapper" tool is included in each PEPscreen® shipment to aid in this process. Add 400 µL (or 2 x 200 µL) of 50% (v/v) ACN/H₂O solution to each tube. For sets containing mostly hydrophobic peptide sequences, add 200 µL of ACN, followed by 200 µL of H₂O.



- Recap the tubes, ensuring the cap is tightly sealed. Incomplete capping can result in solvent leakage from the tubes. It is critical the caps and tubes not be interchanged to prevent cross-contamination of the samples. If there is any doubt as to which caps belong to which tubes, we recommend you wash and dry all the caps before recapping or use new caps. Cross-contamination of peptides can lead to invalid results.
- Return the capped tubes to the rack. Apply the rack lid and secure by locking the lid. When the lid is "locked," invert the rack several times, allowing dissolution to take place.
- The capped tubes can be removed from the rack for inspection. Gentle tilting of the tube will determine whether or not the peptides have dissolved.
- Continue the inspection for each tube, making note of the peptides not completely dissolved. Sonication may help in dissolving the peptides. If sonication does not completely dissolve the peptides, you may want to explore the strategies outlined in steps 6 and 7.
- If there are several peptides that have not dissolved, add additional aliquots of the ACN/H₂O mixture and repeat the mixing and sonication steps. A volume of up to 0.7 mL is practical for use in the tubes supplied. If there are still peptides that are not dissolved, follow the procedure outlined in step 7.
- Label a 10 mL polypropylene tube corresponding to each undissolved peptide. Remove the cap from the corresponding tube of undissolved peptide and transfer the contents of the tube into the labeled 10 mL polypropylene tube. Lyophilize the 10 mL tubes to remove the solvent. DMSO is likely the most suitable solvent for hydrophobic peptides. After lyophilization, add a minimum volume of solvent (typically 100 μ L of DMSO) and dilute the solution with a 50/50 (v/v) ACN/H₂O mixture to a total volume of 400 μ L. This will maintain an equivalent peptide concentration for all of the peptides in the set. Vortexing the sample may assist in dissolving the peptide. 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 rack.

It is important to take a sensible approach when working with peptide sets. Although some peptides may not be completely dissolved, there will likely be enough dissolved peptide to provide meaningful results in many applications, such as binding assays, where an excess of peptide is typically present. Peptide solutions containing DMSO must be frozen when not in use to prevent oxidation of cysteines and methionines in the peptide sequence.

Other Solvent Choices

Other solvent mixtures for dissolving peptides before final lyophilization are different combinations of acetic acid/acetone/nitrile/water mixtures. The acetic acid and the acetonitrile concentrations should range from 5 to 20%, depending on the relative hydrophobicities of the peptides. When high concentrations of acetic acid are used (>10%), the peptides should be lyophilized or frozen quickly to avoid potential hydrolysis reactions.

If using DMSO, it is beneficial to add water to the DMSO (e.g., 20%) to assist hydration of highly hydrophilic peptides. Since DMSO is a weak oxidizing agent, peptides containing cysteine (C), methionine (M), or tryptophan (W) can be oxidized. For this reason, DMF or N-1-methyl-2-pyrrolidone (NMP) may be better choices for dissolving your peptide set.

Handling Guidelines for PEPscreen® Peptide Sets

The dissolved peptides constitute a set of stock peptide solutions. This peptide set should be handled with much care, avoiding the possibility of cross-contamination within the peptide set.

Making replicate sets from the stock peptide set is useful for minimizing repetitive freeze-thaw cycles. Aliquotting can be done into multi-well plates or into a set of capped polypropylene tubes (as originally supplied). It is recommended that you uncap individual tubes or, at the most, one row of tubes at a time to reduce the possibility of cross-contamination to your stock set of peptides. Many applications will require dilution from the stock peptide set.

The stock and replicate sets should be stored at -20 °C or colder. It is not recommended to keep peptides in a liquid state for long-term storage. Peptides in solution may degrade by chemical processes or grow microbiological contaminants, resulting in an unusable sample. For long-term storage, the peptides are much more stable when stored in a lyophilized state.

If liquid state storage is unavoidable, we recommend adding 0.1% (w/v) sodium azide, which serves as a preservative and eliminates microbial growth in the solution.

Conclusion

This document serves as a general guideline for working with PEPscreen® peptide sets. Because each peptide set is unique, it may be necessary to tailor special methods for dissolving your peptide set. If you have specific questions regarding your peptide set, please email us at techsrv@sigma.com.

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