Lot Variability

Peptide content

This is determined by amino acid analysis and should not be confused with peptide purity. Peptide content refers to the percentage of all the peptides present relative to the rest of the non-peptide impurities, INCLUDING moisture. This is also a big source of variability. Peptides can have anywhere from 10% to 90% water content, depending on the amino acid composition. Hydrophilic peptides have a tendency to bind more water. In addition, lyophilization conditions (original sample volume, solvent composition, sample load in the lyophilizer, length of lyophilization, etc.) can also affect the moisture content of the final product. Our standard lyophilization of at least 48 hours removes most of the free moisture, but there can still be differences in the amount of bound water especially in very hydrophilic peptides. It is for this reason that it is important for the end user to measure the peptide concentration in the peptide SOLUTIONS, if possible, to ensure that peptide activities are expressed based on the same amounts of peptide.

Peptide Purity

This refers to the amount of correct peptide product relative to all other impurities, EXCEPT moisture, and is determined by analytical HPLC. Peptide purity is the most common variability, especially in peptides ordered at relatively low purity (below 80%). For example, if a peptide is ordered at 80% purity, the peptide shipped may be 80% to 100% pure, depending on what is obtained from the synthesizer or the purification step. The lot-to-lot variability becomes even greater the lower the peptide purity ordered. Therefore, if the customer bases their experiments on peptide weights, the actual amount of correct peptide present might vary. In addition, the nature of the impurities may also be important. It is important to remember that in some cases, peptide impurities with one or few amino acid deletions can still be active and contribute to the overall activity of the peptide. Obviously, the higher the purity of the peptide, the lesser the variability.

Aggregation

For peptides that tend to aggregate heavily (hydrophobic peptides), the peptide activity can greatly depend on which aggregate forms are still active. Some aggregates are more soluble and active than others. The degree of aggregation can depend on the peptide concentration. The higher the concentration, the higher the possibility for the peptides to aggregate.

Chemical transformation

During transit or storage, the peptide can undergo a series of chemical transformations that may lead to inactivity. Common examples are peptides containing Asp-Gly in their sequences that lead to iso-Asp formation (usually inactive), cross-linking of Cysteine containing peptides through the formation of disulfide bonds, oxidation of methionine to sulfoxides and sulfones, oxidation of tryptophan, pyroglutamate formation, etc. For more information go to Peptide Stability.

Non-peptide impurities

Some impurities in the peptide may be toxic to cells, if these peptides are used for cell culture studies. Dithiothreitol (DTT) may be tolerated at less than 1μM concentration in most cells. For crude peptides that go through the common ether precipitation process, the residual amount of DTT should be relatively small. For peptides that have gone through a purification step, the peptide is generally free of DTT.

In addition, most peptides are purified in the presence of trifluoroacetic acid (TFA) in the solvent and the amount of residual TFA and TFA salts in the peptide are difficult to quantify. Free TFA can generally be removed after lyophilization for at least 48 hours, but TFA salts with the peptide or with other buffer ions may be difficult to completely remove. If TFA or TFA salts are suspected to cause problems in the experiment, the customer should request specific buffers be used in the final purification and lyophilization steps.