FAQ

How should I store and handle my synthesized peptides?

Peptides are shipped at room temperature, and are highly stable at lyophilized form in sealed bags. Peptides should not be kept in solution for long periods of time.

- Peptide storage guidelines: For long-term storage, peptides should be stored in lyophilized form at -20°C, or preferably at -80°C with desiccant in sealed containers to minimize peptide degradation. Under these conditions, peptides can be stored for up to several years. This type of storage prevents bacterial degradation, oxidation, and the formation of secondary structures.

- Opening the package: It is better to equilibrate the peptides to room temperature in a desiccator prior to opening and weighing. Failure to warm the peptides beforehand can cause condensation to form (peptides tend to be hygroscopic) on the product when the bottle is opened. This will reduce the stability of the peptides. Before reconstitution, centrifuge the vial of lyophilized peptide at 12,000 x g for 20 seconds. This will help pellet the entire peptide sample for reconstitution.

- Weighing peptides: Weigh out your required quantity of peptide rapidly and store all unused peptide at -20°C or below. Sequences that contain cysteine, methionine, tryptophan, asparagine, glutamine, and N-terminal glutamic acid will have a shorter shelf life than other peptides.

How should I dissolve peptides?

The solubility of a peptide is determined mainly by its polarity. Acidic peptides can be reconstituted in basic buffers, whereas basic peptides can be dissolved in acidic solutions. Hydrophobic peptides and neutral peptides that contain large numbers of hydrophobic or polar uncharged amino acids should be dissolved in small amounts of organic solvent such as DMSO, DMF, acetic acid, acetonitrile, methanol, propanol, or isopropanol, and then diluted using

water. DMSO should not be used with peptides that methionine or free cysteine because it might oxidize the side-chain.

Test a portion of the synthesized peptide before dissolving the rest of the sample. Lyophilized peptides should be centrifuged briefly to pellet all the material. You might need to test several different solvents until you find the appropriate one. Sonication can be used to enhance solubility.

- First, assign a value of −1 to each acidic residue (Asp [D], Glu [E], and the C-terminal –COOH). Next, assign a value of +1 to each basic residue (Arg [R], Lys [K], His [H], and the N-terminal -NH2), and then calculate the overall charge of the peptide.
- If the overall charge of the peptide is positive, the peptide is basic. Try to dissolve the peptide in distilled water if possible. If it fails to dissolve in water, then try to dissolve the peptide in a small amount of 10–25% acetic acid. If this fails, add TFA (10–50 µl) to solubilize the peptide, and then dilute it to your desired concentration.
- If the overall charge of the peptide is negative, the peptide is acidic. Acidic peptides might be soluble in PBS (pH 7.4). If this fails, add a small amount of basic solvent such as 0.1 M ammonium bicarbonate to dissolve the peptide, and then add water to the desired concentration. Peptides that contain free cysteines should be dissolved in de-gassed acidic buffers because thiol moieties will be oxidized rapidly to disulfides at pH >7.

- If the overall charge of the peptide is 0, the peptide is neutral. Neutral peptides usually dissolve in organic solvents. First, try to add a small amount of acetonitrile, methanol, or isopropanol. For very hydrophobic peptides, try todissolve the peptide in a small amount of DMSO, and then dilute the solution with water to the desired concentration. For Cys-containing peptides, use DMF instead of DMSO. For peptides that tend to aggregate, add 6 M guanidine, HCl, or 8 M urea, and then proceed with the necessary dilutions.

To prevent or minimize degradation, store the peptide in lyophilized form at −20°C, or preferably −80°C. If the peptide is in solution, freeze-thaw cycles should be avoided by freezing individual aliquots.

- Positively charged residues: K, R, H, and the N-terminus
- Negatively charged residues: D, E, and the C-terminus
- Hydrophobic uncharged residues: F, I, L, M, V, W, and YUncharged residues: G, A, S, T, C, N, Q, P, acetyl, and amide

Examples:

RKDEFILGASRHD: (+5) + (-4) = +1 This is a basic peptide. See step #2 above.

EKDEFILGASEHR: (+4) + (-5) = -1 This is an acidic peptide. See step #3 above.

AKDEFILGASEHR: (+4) + (-4) = 0 This is a neutral peptide. See step #4 above.

Can I predict whether a peptide will be soluble?

The solubility of a peptide in water cannot be predicted by studying its structure. However, the ε-amino group of Lys and the guanidine of Arg are usually helpful for estimating the solubility of peptides, particularly those with short sequences. In contrast, acidic peptides that contain Asp and Glu tend to be insoluble in water, but can be dissolved easily in dilutedammonia or basic buffers.

Certain basic characteristics can be used to predict solubility:

- Peptides containing <5 amino acids are commonly soluble in aqueous solutions. However, if the entire sequence consists of hydrophobic residues it will have only limited solubility or could be completely insoluble.
- Hydrophilic peptides that contain >25% charged amino acids (E, D, K, R, and H) and <25% hydrophobic residues are usually soluble in aqueous solutions.

- Hydrophobic peptides whose sequence contains ≥50% hydrophobic residues might be completely or only partially soluble in aqueous solutions. These peptides should instead be dissolved in organic solvents such as DMSO if they do not contain C, W, or M residues. If they do contain these amino acids, they should be dissolved in DMF, acetonitrile, isopropyl alcohol, ethanol, acetic acid, 4–8 M guanidine hydrochloride (GdnHCl), or urea prior to being diluted carefully in aqueous solution.

- Hydrophobic peptides that include >75% hydrophobic amino acids are generally not soluble in aqueous solutions. Instead, very strong solvents such as TFA or formic acid must be used for the initial solubilization. However, the peptide might precipitate when added to an aqueous buffered solution. As such, high concentrations of organic solvents or denaturants might be needed to dissolve these peptides.

- Peptides that include a very high proportion (>75%) of D, E, H, K, N, Q, R, S, T, or Y can form intermolecular hydrogen bonds (cross-links), which can result in gel formation in concentrated aqueous solutions. Therefore, peptides should be dissolved in an organic solvent that is compatible with the final experiment. After dissolving the peptides in organic solvent, the solution should be added slowly (dropwise) to a stirring aqueous buffered solution. The limit of solubility is reached when the resulting peptide solution begins to show turbidity.

How do I choose the best level of peptide purity for my research?

Crude peptides are not recommended for biological assays. Crude peptides may contain large amounts of non-peptide impurities such as residual solvents, scavengers from cleavage, TFA and other truncated peptides. TFA cannot be totally removed. Peptides are usually delivered as TFA salt. If residual TFA is a problem for your experiment, we recommend other salt forms such as acetate and hydrochloride. These salt forms are usually 20-30% more expensive than the regular TFA salt. This is due to the peptide loss that takes place during the salt conversion and the greater amounts of raw materials required.

Priveel Custom Peptides recommends the following levels of peptide purity for various projects:

>70% purity

- Peptide arrays
- Antigens for antibody production
- Competitive elution chromatography
- ELISA standards for measuring antisera titers

>80% purity

- Western blotting studies (non-quantitative)
- Enzyme-substrate studies (non-quantitative)
- Peptide blocking studies (non-quantitative)
- Affinity purification
- Phosphorylation assays
- Protein electrophoresis applications and immunocytochemistry

>95% purity

- ELISA standards and RIA protocols (quantitative)
- Receptor-ligand interaction studies (quantitative)
- In vitro bioassays and in vivo studies
- Enzyme studies and blocking assays (quantitative)
- NMR studies
- Mass spectrometry
- Other quantitative assays

>98% purity

- SAR Studies
- Clinical trials
- APIs (Active Pharmaceutical Ingredients)
- Commercial products
- X-ray crystallography studies
- Other sensitive experiments: enzyme-substrate studies, receptor-ligand interaction studies, blocking and competition assays

What is peptide purity?

Peptide purity is the amount of the target peptide as determined by HPLC at 214 nm, where the peptide bond absorbs. Water and residual salts are not detected by UV spectrophotometer. Other impurities that can be found in the content include deletion sequences (shorter peptides lacking one or more amino acids of the target sequence), truncated sequences (generated by capping steps to avoid the formation of deletion peptides), and incompletely deprotected sequences (generated during the synthesis or the final cleavage process).

Peptide purity does not include any water or salts in the sample. TFA results from HPLC purification. The free N terminus and other side chains such as Arg, Lys, and His form trifluoroacetates and this allows small amounts of TFA to contaminate the peptides. Peptides are usually delivered as trifluoroacetates containing residual water. Even in lyophilized peptides, varying amounts of noncovalently bound water still exist.

What are other substances (impurities) in the peptides?

Impurities	Non-Purified Peptides	Purified Peptides (HPLC)
Deletion sequences1	✓	✓
Truncation sequences2	✓	✓
Incompletely deprotected sequences3	✓	✓
Sequences modified during cleavage4	✓	✓
DTT (dithiothreitol)	✓	×
TFA (trifluoroacetic acid)	✓	✓
Acetic acid	✓	×
Peptides that have undergone side reactions such as proline isomerization or isoaspartimide formation, etc.	✓	✓

The impurities in non-purified peptides are both peptides and non-peptides, the impurities in purified peptides are mostly peptides with modified sequences, except for TFA salt.

- Shorter peptides lacking one or more amino acids of the target sequence
- Generated by capping steps to avoid the formation of deletion peptides
- Generated during the synthesis or the final cleavage process
- Reattachment of protecting groups at other locations on the peptide

What is net peptide content?

The net peptide content is different from the peptide purity. The net peptide content is the percentage of peptides relative to nonpeptidic materials, mostly counterions and moisture. The net peptide content can be determined by amino acid analysis. Please place a request for a quote if you require this service. Usually, hydrophilic peptides absorb tiny amounts of moisture even under strict lyophilization conditions. Net peptide content may vary from batch to batch depending on the purification and lyophilization processes.

What salt form should I use?

Peptides are usually delivered as TFA salts. If residual TFA would be problematic for your experiment, we recommend other salt forms such as acetate and hydrochloride. These salt forms are usually 20-30% more expensive than the regular TFA salt because of the peptide loss that takes place during the salt conversion and the greater amounts of raw materials required.

How are peptides synthesized?

Unlike the natural protein synthesis, peptides are synthesized from the C to N terminus. At Priveel Custom Peptides, peptide synthesis is performed using PeptideSyn technology based on Fmoc or t-Boc chemistry to protect the alpha amino group. The deprotection agent (piperidine for Fmoc, TFA for Boc) frees the alpha amino group in preparation for coupling the next amino acid in the sequence. This reveals a new N-terminal amine to which the next amino acid may be activated by one of several reagents, forming a peptide bond. When the synthesis is complete, peptides are cleaved from the resin and de-protected. Peptides are then precipitated, washed, and lyophilized.

What are your QC standards for peptide synthesis?

All materials supplied to Priveel Custom Peptides are considered the confidential property of the customer. Priveel Custom Peptides provides free HPLC and MS results with your package. Peptides are purified by reverse-phase chromatography. The chromatogram indicates the number and

relative amount of by-products. The molecular mass of the peptide is determined by mass spectrometry to confirm that the correct product is being delivered. MS results also show the masses of the main impurities. Additional analysis revealing net peptide content can be performed upon request. Net peptide content is indicated by either amino acid analysis or elemental analysis. These methods allow the verification of the amino acid composition of the peptides. They serve as additional means of confirmation of peptide identity. All synthetic peptides meeting the customer's purity criteria are sent. All residual materials, such as peptides not meeting the customer's purity criteria are discarded. These residual materials can be sent to the customer upon request.

Can you aliquot my peptides?

Upon request, Priveel Custom Peptides can aliquot part or all of your order into smaller quantities for a minimum fee of $1 per tube. Aliquoted products are more expensive but may save you time, effort and money during determination of peptide solubility. Your peptides will also be more stable because they will not be exposed to as many freeze-thaw cycles, as many openings and closings of the container, mishandling, or bacterial contamination. Peptide oxidation, degradation, and aggregation are less prevalent in aliquoted samples.

What are APIs, catalog peptides and custom peptide synthesis?

APIs (active pharmaceutical ingredients) are the substances in drugs that are pharmaceutically active, such as oxytocin acetate, enfuvirtide acetate, and so on. Catalog peptides are commercially available sequences. They are usually produced in bulk at high levels of purity. These peptides are usually customized to customers' specific requests. For example, specific sequences, modifications, purity levels, or lengths may be required by the customer. The turnaround time for most API peptides is 2-3 weeks.

What is the minimum quantity for one order?

The minimumal quantity to be ordered should be at leastis 1 mg. At Priveel Custom Peptides, There there is no maximum upper limit at Priveel Custom Peptides for research and or GMP peptides.

What is the maximum peptide length that Priveel Custom Peptides can produce?

Priveel Custom Peptides has synthesized a peptide of 200 amino acids in length. Peptides of 50 amino acids are synthesized routinely.

What is solid-phase synthesis?

Organic reactions are carried out on substrates covalently attached to a polymeric resin. Solid-phase synthesis can be better than the traditional synthesis because the overall reaction takes place much more quickly, the process can be automated with robots, and synthetic intermediates do not need to be isolated because reagents are washed away during each step.

What are resins and linkers?

Resin is the polymeric backbone to which substrates are anchored. Different resins have different properties. For example, polystyrene swells in non-polar solvents, while polyethylene glycol swells in polar and non-polar solvents. Linkers are intermediate structures that attack the resin to the substrate. Different linkers can be used to unmask different functional groups on the substrate.

What is a protecting group?

Protecting groups are fragments that binds to functional groups and blocks their reactivity. Some are acid-labile protecting groups such as Boc and tert-Bu ester. Some are base labile protecting groups such as Fmoc and Fm ester. Some others are fluoride-labile protecting groups such as Tmsec and Tmse ester. To ensure specific coupling between the required carboxyl and amino groups, the protecting groups should be easy to attach and remove without changing the rest of the peptide.

What are acetylation and amidation?

Chemically synthesized peptides carry free amino and carboxy termini. The need for N-terminal acetylation or C-terminal amidation must be stated explicitly during ordering. It is impossible to perform these modifications after synthesis has been completed.

N-terminal acetylation and C-terminal amidation reduce the overall charge of a peptide and decrease solubility. However the stability of the peptide usually increases because the terminal acetylation and amidation allow the peptide to mimic the native protein more closely. In this way, these modifications may increase the peptide's biological activity.

Is a spacer required for fluorescent modification?

Usually, dyes such as biotin and FITC can be introduced either N-terminally or C-terminally. We recommend N-terminus modification for its higher success rate, shorter turnaround time, and ease of operation. Peptides are synthesized from the C-terminus to the N-terminus. N-terminus modification is the last step in the SPPS protocol. No more specific coupling steps are required. In contrast, the C-terminus modification requires additional steps and is usually more complex.

Most dyes are large aromatic molecules. The incorporation of such bulky molecules may help to avoid interactions between the label and the peptide. This will help maintain peptide conformation and biological activity. It is recommended that a flexible spacer such as Ahx (a 6 carbon linker) be included to render the fluorescent label more stable. Otherwise, FITC could easily link to a cysteine thiol moiety or the amino group of lysine at any position.

How to calculate the peptide concentration?

Peptide purity is the term used to describe the percentage of peptide with the target sequence among the total quantity of material. Because peptide bond

formation is not 100% efficient during peptide synthesis, not all polypeptide chains are made of the target sequence. For example, some chains might not be complete, or amino acids might not bind appropriately. These deleted or incorrect sequences form a certain percentage of peptides in most peptide mixtures. We analyze and purify crude peptides using reverse phase HPLC, and then analyze the resulting material using MS to achieve the desired target sequence purity.

After your peptide has been purified and lyophilized, the white peptide powder will contain some non-peptide components such as water, salts, absorbed solvents, and counter ions. The peptide content describes the actual percentage weight of the peptide in your final product. This number varies, but is commonly 50–90% depending on the purity, sequence, and methods used for synthesis and purification. When calculating the concentration of peptide solutions for biological assays or other experiments, it is essential that the peptide content is accounted for. The actual peptide concentration can be determined by subtracting the non-peptide weight from the total weight, which allows you to determine what volume of solvent to use. For example, if you were using 1 mg of final product to make a 1-mg/ml peptide solution with a content of 80%, you would use 800-µl of solvent rather than 1000 µl.

It is important to note that peptide content and peptide purity are two distinct measurements. Purity is determined using HPLC, and revealed the presence or absence of contaminating peptides with the incorrect sequences. In contrast, the net peptide content provides only information regarding the percent of total peptide vs. total non-peptide components: it does not consider the presence of multiple peptides. The net peptide content can be determined accurately by performing amino acid analysis or UV spectrophotometry.

It is difficult to determine the actual concentration of a peptide based on the weight of the lyophilized peptide. Lyophilized peptides might contain 10–70% water and salts by weight. Generally, hydrophobic peptides contain less bound water and salts than do hydrophilic peptides.

If the peptide has a chromophore in its sequence (W or Y), the peptide concentration can be determined conveniently using the extinction coefficient of these residues as follows:

- Molar extinction coefficients of chromophoric residues at 280 nm at neutral pH using a 1-cm cell:
  Tryptophan 5560 AU/mmole/ml
  Tyrosine 1200 AU/mmole/ml
- The molar extinction coefficient of chromophoric residues is measured at 280 nm at neutral pH using a 1-cm cell. That of W is 5560 AU/mmole/ml, whereas that of Y is 1200 AU/mmole/ml
- The extinction coefficient of the chromophores in a peptide sequence is generally additive; therefore, the overall molar extinction coefficient of the peptide depends on the type and number of the choromophoric residues in the sequence.
- When performing the calculations, the mg peptide per ml = (A280 x DF x MW) / e, where A280 = the actual absorbance of the solution at 280 nm in a 1-cm cell, DF = dilution factor, MW = molecular weight of the peptide, and e = molar extinction coefficient of each chromophore at 280 nm.
- Hypothetical example: A 50× diluted solution of peptide with the sequence GRKKR RQRRR PPQQW DCDLY RPYEK T (MW = 3418) would measure 0.5 AU at 280 nm in a 1-cm cell. The concentration of the original peptide in the stock peptide solution would be calculated as follows:mg peptide/ml = (0.5AU x 50 x 3418 mg/mmole) / [(1 x 5560) + (2 x 1200)] AU/mmole/ml = 10.7
- Cautions:Any absorbance calculation assumes that a peptide is unfolded and that the chromophores are exposed. This is generally an acceptable assumption for short, soluble peptides. If there are doubts about the solubility or folding of a given peptide, it is advisable to perform measurement under denaturing conditions (e.g., in the presence of 6 M GdnHCl or 8 M urea). It is important to remember that these peptide solutions will not be functional until the denaturants have been removed.If

the sequence does not have W or Y amino acid, the peptide concentration can only be determined using amino acid analysis.

How do I Detect Small Peptides using SDS-PAGE?

If your sample contain proteins of interest that are <20 kDa, please download a protocol that explains how to detect synthetic peptides using SDS-PAGE, including effective methods for Coomassie blue staining, silver staining, and electroblotting.

Tricine-based SDS-PAGE is used most commonly to separate proteins sized 1–100 kDa, and is the electrophoretic system of choice for resolving proteins <30 kDa. Although visualizing small peptides using SDS-PAGE is challenging, Tris-tricine gels afford better resolution. However, if you simply want to detect the peptide, MS remains the most accurate method for confirming the identity of a peptide.

Small peptides binds to Coomassie brilliant blue less readily that do larger proteins. Therefore, smaller peptides are difficult to detect using Coomassie or silver staining. Additional sample could be loaded to allow peptides to be visualized on gels; changing the percentage of the gel will only help if you think that your peptide migrated out gel. In this instance, the percentage of cross linker in a regular 17% gel could be increased, and the pH of the resolving gel could be increased to 9.5 (compared with the normal 8.8). Finally, the addition of 4–8 M urea helps sharpen bands.

The use western blotting rather than gel staining is a far more sensitive detection method. However the peptide might simply pass through the membrane during transfer. If you think this occurs, the experiment can be repeated using two pieces of membrane and shorter transfer time (<1 hour at 200 mA). A membrane with a 0.2-µm pore size should be sufficient: although smaller pore sizes are available, they should not be necessary. An additional option would be to try semi-dry transfer for 15–20 minutes using the current density (mA/cm2) recommended for the apparatus. A short transfer time of 15

min works for most small peptides. If it is possible to plan ahead, a control small peptide labeled with biotin could be synthesized to monitor the transfer process and assess the ability of the peptide to bind to the membrane using streptavidin-conjugated HRP.

How do I dissolve peptides in DMSO?

Dimethyl sulfoxide (DMSO) is an organosulfur compound with the formula (CH3)2SO. DMSO is used frequently in cell banking applications as a cryoprotectant because it prevents intracellular and extracellular crystals from forming in cells during the freezing process. For most cryopreservation applications, DMSO is used at a concentration of 10%, and is usually combined with saline or serum albumin.

Hydrophobic peptides can be dissolved easily in DMSO. However, peptides in DMSO might be cytotoxic to cells, even though DMSO increases cell permeability. High concentrations of DMSO should never be used for cell culture. 5% is very high, and will dissolve the cell membranes. Most cell lines can tolerate 0.5% DMSO, and some cells can tolerate up to 1% without severe cytotoxicity. However, primary cell cultures are far more sensitive. Therefore, if you are using primary cells a dose-response curve (viability) should be performed using DMSO concentrations <0.1%.

Try to dissolve very hydrophobic peptides in a small amount of DMSO (30–50 µl, 100%), and then slowly add the solution drop-wise to a stirring aqueous buffered solution such as PBS (or your desired buffer) to the required concentration. If the resulting peptide solution begin to show turbidity, you have reached the limit of solubility. Sonication will help dissolve the peptides.

Rule of thumb:

- 0.1% DMSO is considered to be safe for almost all cells.
- A final concentration of 0.5% DMSO is used widely for cell culture without cytotoxicity.

- 1% DMSO does not cause any toxicity in some cells, but 0.5% DMSO is recommended.
- 5% DMSO was used successfully in some cells.To maintain a final concentration of 0.5%, you can make 200x stock in 100% DMSO.

How are my peptides shipped?

All peptides are shipped directly to the end-user from our Chennai office by Blue Dart/ Professional/ Speed Post for domesitc service and FedEx for international service. On the day of the shipment an e-mail is sent to the end-user confirming the shipment and providing a tracking number in order to track the package if needed.

Each peptide is shipped at ambient temperature in vacuum-sealed bags(to avoid moisture-based oxidation) . Various tests have revealed shipping under these conditions has no impact on the viability and shelf-life of the peptides. However, upon receipt of the product by the end user, it should be stored immediately at -20 C or lower.