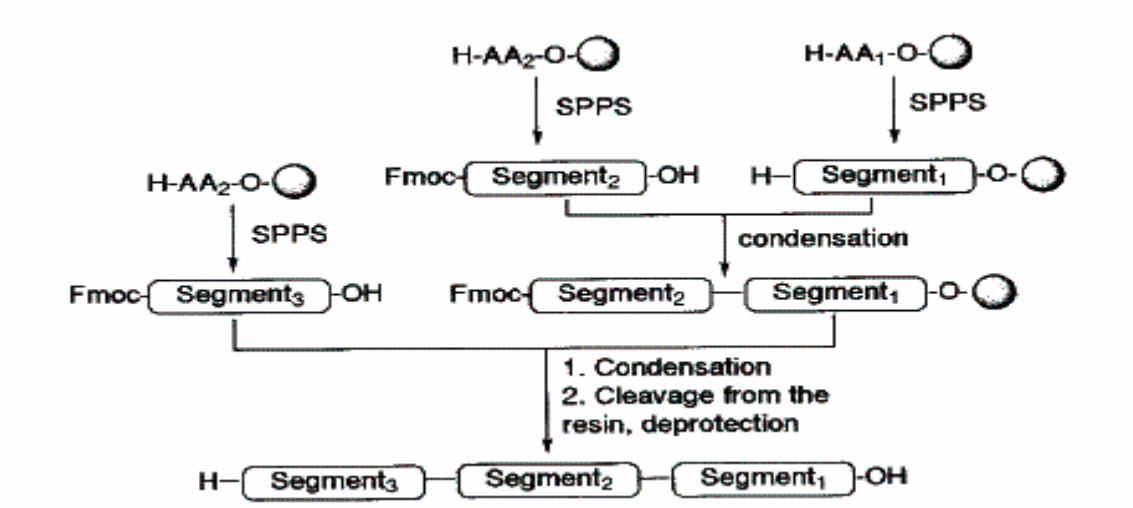
1. 如何进行多肽合成？

Fmoc and Boc methodologies are both employed, using solid and solution phase reactions. Fmoc chemistry is most suitable for simple peptides and sequences prone to oxidation, but the use of Boc chemistry allows us to synthesise difficult sequences, and gives a greater flexibility in the synthesis of modified peptides. Most peptide synthesis is carried out in the solid phase, but certain modifications are carried out in solution after peptide synthesis and cleavage from the resin.

2. Fmoc 法固相合成的基本流程是如何进行的？

详细流程如下图：



3.什么样的肽端（C- and N-terminal endings）比较好?

This depends on the application you have in mind for your peptides. It may be a good idea to choose the terminal ends of the peptide dependent on the natural occurrence of the sequence: 1. The peptide(s) should mimic an internal sequence of a protein. The peptide(s) should not be charged at the ends. The N-terminus of the peptide(s) should be acetylated and the C-terminus should be an amide. 2. The peptide sequence is the C-terminal end of a protein. The C-terminus should be the free acid and the N-terminus should be acetylated. 3. The peptide sequence is the N-terminal end of a protein. The C-terminus should be an amide and the N-terminus should be in the natural free amine form. • For cytotoxic T-cell epitope studies the peptides should have both, a free amino group at the N-terminus and a free acid at the C-terminus. These ends are the natural equivalents to the peptide fragments, processed intracellularly from whole proteins. • Peptides with acetylated N-terminus and an amide as C-terminus are more resistant to exopeptidases, which may be an important factor regarding the time a peptide will be functional in a biological assay. • Biotinylated peptides can be useful in combination with streptavidin coated surfaces (e.g. beads, plates or

microarrays). • Peptides with a thiole group (e.g. cysteine) can be directly bound to gold surfaces or to amino groups by the use of bifunctional crosslinkers.

4.如何保存多肽？

The lyophilized peptides should be kept in a cool, dark place. Long term storage of the peptides should be done in a freezer at -20 to -80°C. Most peptides stored in this way will remain stable for several years. Peptides in solution are not as stable as in the lyophilized form. Peptide solutions should be neutral to slightly acidic (pH 5-7) and stored frozen at -20°C. To avoid repeated freeze-thaw cycles it is recommended to divide the stock solution into aliquots. • maintain sterile conditions • Cys, Met and Trp residues tend to oxidize (oxidation-rate increases with pH)

5.如何处理多肽的溶解问题？

The solubility of peptides is strongly dependent upon the peptide sequence. For some very hydrophobic sequences it may not be possible to use aqueous solutions without any additional organic solvents. 1. Try to dissolve the peptides in sterile water with sonication (1-10 mg peptide/ml). 2. If this fails, add acetic acid up to a total concentration of 10% (v/v) for basic peptides or aqueous ammonia for acidic peptides and sonicate. (Count the number of basic (R, H, K and free N-terminus) and acidic residues (D, E and free C-terminus) of the peptide) Examples: Ac-HN-RFREQIVKPFK-CONH2 -> 0-1+0-1+1+0+0+0-1+0+0-1+0 = -3 -> basic peptide H2N-FVQADIDYIT-COOH -> -1+0+0+0+0+1+0+1+0+0+0+1 = +2 -> acidic peptide 3. For peptides that remained insoluble add organic solvents such as acetonitrile, DMSO or DMF up to a concentration of 20% (v/v). NOTE: The use of organics such as acetonitrile, DMF, DMSO etc. may interfere with some biological assays. If DMSO is used, peptides with Cys, Met and Trp oxidize faster!

6.如何经行多肽的溶解实验？

多肽的溶解度取决于多肽序列中所包含的氨基酸及不同的基团，实验前要清楚序列所包含的的氨基酸及基团的极性。尝试不同的溶剂来溶解多肽，必要时可超声助溶，但是要注意 超声处理会引起溶液发热和多肽降解。

Amino acids and group classification:

Acidic amino acid : D, E, C-terminus

Alkaline amino acid: K, R, H, N-terminus

Nonpolar hydrophobic amino acids: F, I, L, M, V, W, Y, A, P,

Polarity hydrophilic amino acids: G, S, T, C, N, Q

I. Each assignment of acidic amino acid value of 1, including aspartic acid, glutamic acid (E) (D),

and the carboxyl terminal - COOH. Each basic amino acid assignment for + 1, including arginine, lysine (K) and histidine (R) (H) and amino terminal -- NH2. Then calculate the peptide on the number of charge.

II. If charged by the entire length of peptides is positive, that the peptide is alkaline. Can first try to use distilled water to dissolve; If it does not dissolve in water, then try to use a small amount of 10% 25% acetic acid solution, if it still fails, add some TFA to solubili zation (10 to 50 edged up), and then diluted to the concentration of ideal.
III. If charged by the entire length of peptides were negative, indicated that the peptide is acidic. Acidic peptide can try to dissolve in PBS (PH 7.4), if it is not dissolved, add a s mall amount of alkaline solution, such as 0.1 M ammonium bicarbonate, then add water di lute concentration to ideal. Contains free cysteine peptides should be soluble in acid buffer of degassing, because when the PH > 7, sh will be quickly oxidized to disulfide.

IV. If a charge is zero, the whole period of peptide peptides is neutral. Neutral peptides ar e usually soluble in organic solvents. First of all, try adding a small amount of acetonitrile, methanol or isopropyl alcohol. For highly hydrophobic peptide, can use a small amount of dimethyl sulfoxide dissolved, then diluted to the concentration of ideal. For peptides that c ontain free cysteine, need to use DMF instead of DMSO. For peptide has a tendency to a ggregate, add 6 m, 8 m urea or guanidine hydrochloride, and then make the necessary dilu tion

7.在肽产品中 Net Peptide 是什么意思？

The weight of the lyophilized peptide composes of the peptide and water weight. The water and counter-ion content vary widely, depending on the given peptide sequence. Typically 90% are peptide and the other 10% are water and counter-ions. For some highly charged peptides the water and counter-ion content can increase up to 30%.

8.我定制的多肽纯度是 98%，其它 2%是什么？

The peptide purity gives the percentage of the correct sequence in a given sample. The rest of the sample consists of truncated sequences, deletion sequences or otherwise modified sequences.

9.PSI 能合成包含多少个氨基酸的多肽？

The minimum length of peptides should not be less than 5 amino acids. For shorter peptide sequences cleavage from the synthesis resin and following purification can cause problems. The standard maximum lengths are peptides with up to 60 residues. However, upon request we will synthesize longer peptides (>60 amino acids).

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