Planning Your Peptide-Antibody Projects

PEPTIDE DESIGN

One of the axioms that GenScript respects is "Quality by Design". Using **OptimumAntigen™ design tool**, GenScript's peptide designing process is in line with this particular motto, delivering quality peptide and antibody projects smartly designed right from the beginning.

1. Peptide Purity

For antibody generation and testing, peptide purity > 70% is enough, however, for biological activity studies, peptide purity > 95% is required. GenScript is able to develop peptides with different purity levels and has the ability to synthesize peptides with purity greater than 98%.

2. Peptide Amino Acid Composition

Amino acid composition governs every aspect of the peptide's functionality. Hydrophobic and hydrophilic characteristics are key factors to consider. The peptide should incorporate antigenic amino acids sequence and avoid problematic amino acids such as cysteine, methionine, and tryptophan.

3. Peptide Length

Peptide length governs the level of difficulty when synthesizing peptides. For long and complex peptides, GenScript implements its proprietary recombinant peptide system and has the ability to produce peptides with lengths up to 200 residues. Peptide antigens are recommended to contain 10-15 residues to be used in antibody production.

4. Peptide Solubility

GenScript designs peptides of varied solubility by changing the frequency of occurrence of hydrophobic and hydrophilic amino acids. Hydrophobic amino acid content is suggested to be kept below 50% with at least one charged residue incorporated within every five amino acids. In addition, a single conservative replacement or addition of polar residues to the N- or C-terminus may also improve peptide solubility.

5. Secondary Structure

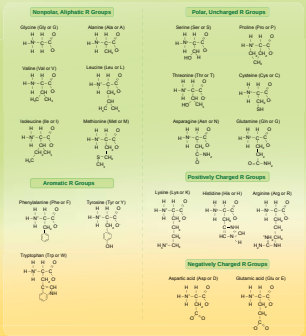
During peptide synthesis, beta sheet formation can cause incomplete solvation of the growing peptide thus deletion sequences in the final product. GenScript sequences avoid multiple or adjacent residues of Val, Ile, Tyr, Phe, Trp, Leu, Gln, or Thr to prevent beta sheet formation. If the above recommendation cannot be implemented practically, conservative replacement may help by reinserting a Gly or Pro at every third residue or replacing Gln with Asp, or Thr with Ser.

PEPTIDE PURITY AND APPLICATION

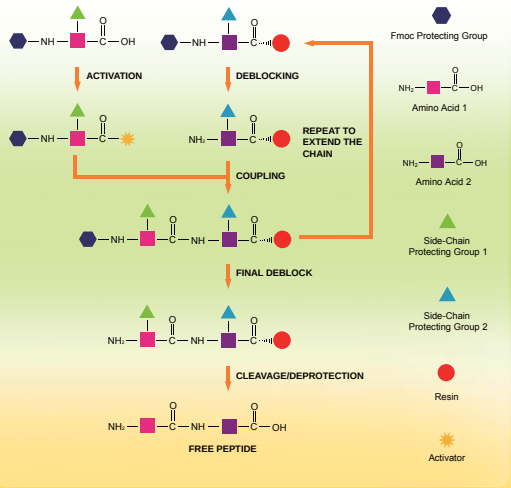
GenScript can synthesize peptides with purity ranging from crude peptide up to 98%. Here is a general guideline for peptide purity requirements:

> 70%	Immunological applications, polyclonal antibody production ELISA tests
> 85%	Semi-quantitative enzyme-substrate studies Phosphorylation studies
> 95%	*In vitro* bioassays such as ELISA, enzymology, biological activity Epitope mapping
> 98%	cGMP peptides for drug studies SAR studies

AMINO ACID STRUCTURES



PEPTIDE SYNTHESIS



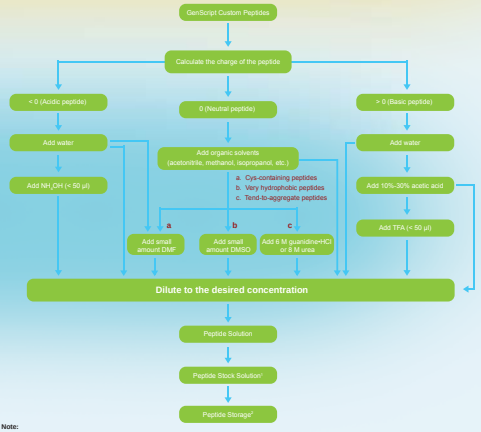
NOMENCLATURE AND PROPERTY OF AMINO ACIDS

Amino Acid	3-Letter Symbol	1-Letter Symbol	M_r	pK_a Values pK_1	pK_2	pK_R	p_I	Hydropathy Index*	Occurrence in Protein (%)
Nonpolar, Aliphatic R Groups									
Glycine	Gly	G	75	2.34	9.60		5.97	-0.4	7.2
Alanine	Ala	A	89	2.34	9.69		6.01	1.8	7.8
Valine	Val	V	117	2.32	9.62		5.96	4.2	6.6
Leucine	Leu	L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile	I	131	2.36	9.60		6.02	4.5	5.3
Methionine	Met	M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R Groups									
Phenylalanine	Phe	F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr	Y	181	2.20	9.11	10.07	5.66	-1.3	3.2
Tryptophan	Trp	W	204	2.83	9.39		5.89	-0.9	1.4
Polar, Uncharged R Groups									
Serine	Ser	S	105	2.21	9.15		5.68	-0.8	6.8
Proline	Pro	P	115	1.99	10.60		6.30	1.6	5.2
Threonine	Thr	T	119	2.09	9.10		5.60	-0.7	5.9
Cystaine	Cys	C	121	1.96	10.28	8.18	5.07	-2.5	1.9
Asparagine	Asn	N	132	2.02	8.80		5.41	-3.5	4.3
Glutamine	Gln	Q	146	2.17	9.13		5.65	-3.5	4.2
Positively Charged R Groups									
Lysine	Lys	K	146	2.18	8.95	10.53	9.74	-3.9	5.9
Histidine	His	H	155	1.82	9.17	6.00	7.59	-3.2	2.3
Arginine	Arg	R	174	2.17	9.04	12.48	10.76	-4.5	5.1
Negatively Charged R Groups									
Aspartic acid	Asp	D	133	1.88	9.60	3.65	2.77	-3.5	5.3
Glutamic acid	Glu	E	147	2.19	9.67	4.25	3.22	-3.5	6.3

* A scale combining hydrophobicity and hydrophilicity of R groups, it can be used to measure the tendency of amino acid to seek an aqueous environment (- values) or a hydrophobic environment (+ values).

PEPTIDE SOLUBILITY GUIDELINES

The solubility of peptides varies depending on its amino acid sequence and modifications.
Solubility test guideline:



Note:

1. It is recommended that the concentration of the stock solution be approximately 1-2 mg of peptide per ml of solution. This is dilute enough to minimize precipitation during storage but concentrated enough to allow relatively small volumes (< 100 µl) of aliquot to be used in assays, thereby minimizing the effects of the solvent.
2. Lyophilized peptides will remain stable for over one year if stored at -20°C. We recommend aliquoting dissolved peptide into tubes for storage at or below -20°C. It is recommended that peptides containing methionine, cysteine, or tryptophan residues be stored in an oxygen-free environment.

OptimumAntigen™ DESIGN TOOL

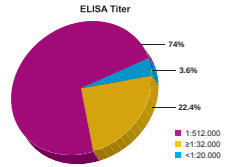
Peptides created through GenScript's OptimumAntigen™ design program have many advantages over full length proteins when it comes to antibody production. Our OptimumAntigen™ Design Tool combines the industry's most advanced algorithms with GenScript's time-tested expertise. Each peptide antigen is measured against several protein databases to confirm the desired antibody and epitope specificity. GenScript stands behind its peptide design and carrier protein conjugation technology. We guarantee the delivery of effective antigens.

Guaranteed Immune Response

GenScript stands behind the promise of its antigen design system.

If peptide antigens were designed, synthesized, and conjugated by GenScript, we guarantee **two positive clones** for monoclonal antibodies or ELISA titer of **1:20,000** or better for polyclonal antibodies developed in any host.

Based on our historical records, at least 96.4% of the orders (pie chart on the right) for which peptide antigens designed, synthesized and conjugated by GenScript received ELISA titer (≥ 1:32,000) higher than guaranteed.



OptimumAntigen™ Benefits

- Strong antigenicity
- Avoid unavailable epitopes due to structural constraints
- Specify desirable cross-reactivity
- Eliminate re-synthesis times with built-in peptide tutorial for synthesis and solubility
- Guaranteed immune response

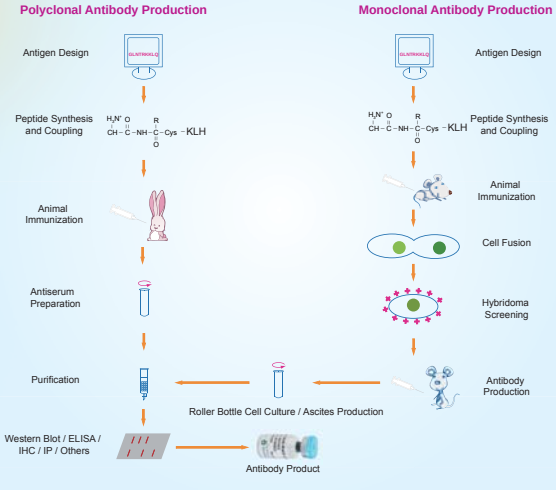
GenScript animal facility is AAALAC International accredited.

Strategies

Our OptimumAntigen™ program searches and assesses your sequence to identify the best candidate peptides that optimize solubility and antigenicity and maximize the chance of synthesis success. Each peptide is compared with our curated BLAST databases of over two dozen species to limit cross-reactivity while improving overall antibody specificity. The following strategies are used for OptimumAntigen™ program:

- Sequence length
- Hydrophilic, surface-oriented, and flexible
- Targeting the N terminus or C terminus
- Continuous versus discontinuous epitopes
- Algorithms
- Coupling strategy
- Experience
- Mechanism learning algorithms

ANTIBODY PRODUCTION



GenScript
The Biology CRO

GenScript USA Inc.
120 Centennial Ave.
Piscataway, NJ 08854, USA

Tel: 1-732-885-9188
Email: info@genscript.com
Web: www.genscript.com

Your Innovation Partner in Drug Discovery!