Avoiding Peptide Assay Failure: Hidden Problems and Solutions

Presented by:

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GenScript
Make Research Easy

Presentation Overview

1. Introduction
2. Key properties of custom peptides
3. Symptoms/Sequence evaluation
4. Solutions Summary & Resources
5. Q&A

About GenScript

Gene Services

Peptide Services

Protein Services

Antibody Services

Discovery Biology Services

Catalog Products

Intrinsic Custom Peptide Properties

1

Contamination

- TFA counter-ions
- Protecting groups
- Truncated sequences
- Deletion sequences
- Endotoxin
- Water

2

Insolubility

- Precipitation
- Aggregation
- Secondary structure formation

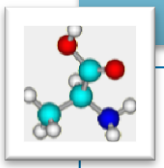
3

Instability

- Oxidation
- Deliquescence
- Colorization
- Deamidation
- Hydrolysis
- Cyclization
- Secondary structure formation

Troubleshooting Peptide Assays

Evaluate Sequences



Examples:

- Calculate net charge
- Identify sequences having propensity for secondary structure formation
- Identify residues that bind contaminants

Identify Symptoms

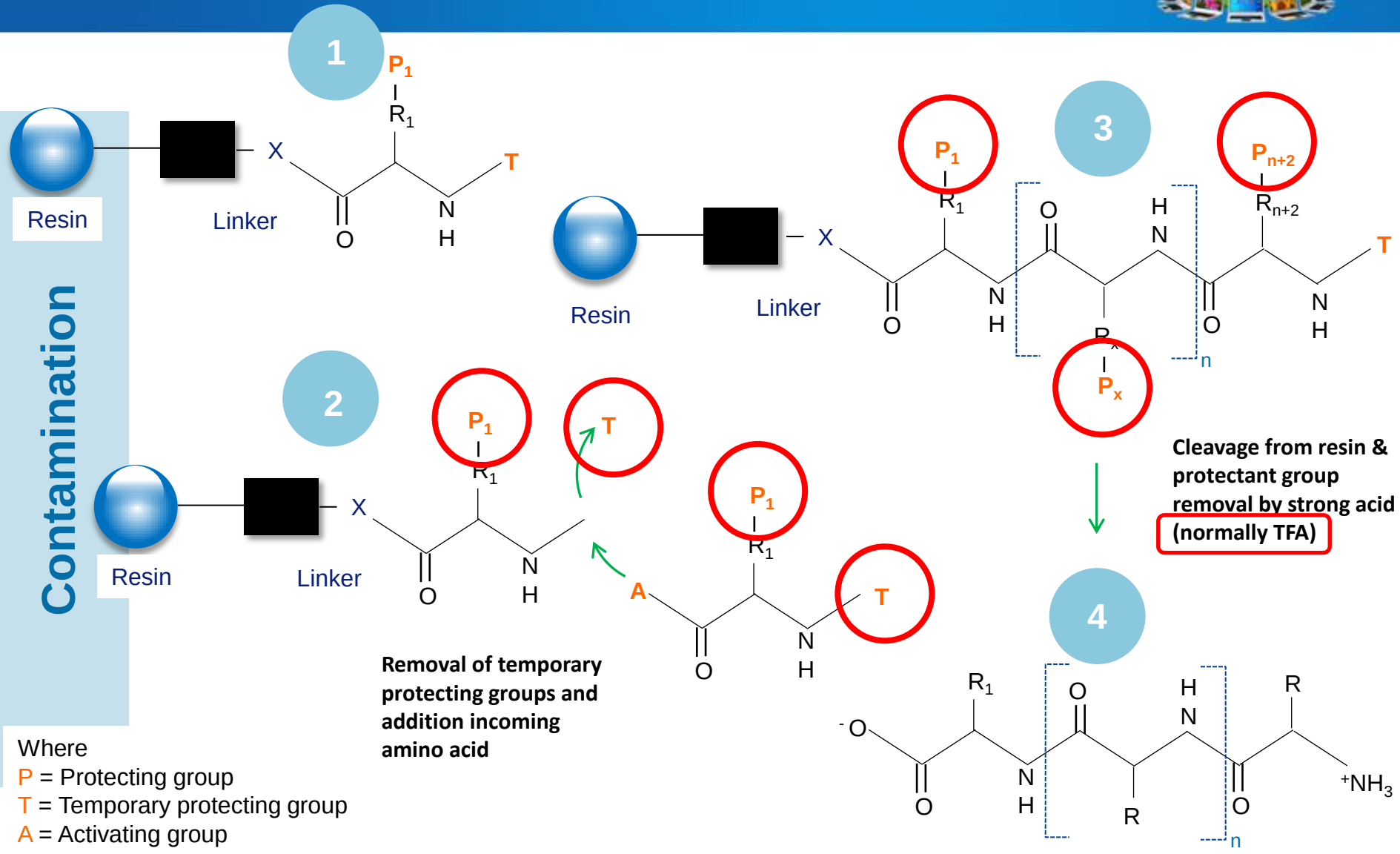
Examples:

- Aberrant cellular or tissue growth
- Aberrant peptide bioactivity
- Abnormal physical peptide characteristics

Employ Solutions

- Pre-emptive sequence optimization
- Peptide preparation
- Assay optimization
- Specialized services

Overview of Solid Phase Peptide Synthesis



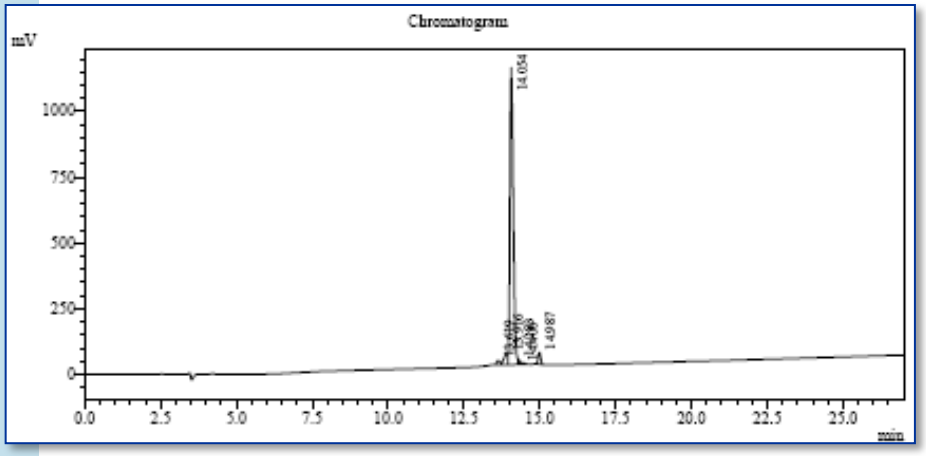
Where
P = Protecting group
T = Temporary protecting group
A = Activating group

Quality Control Analyses

Contamination

HPLC Trace

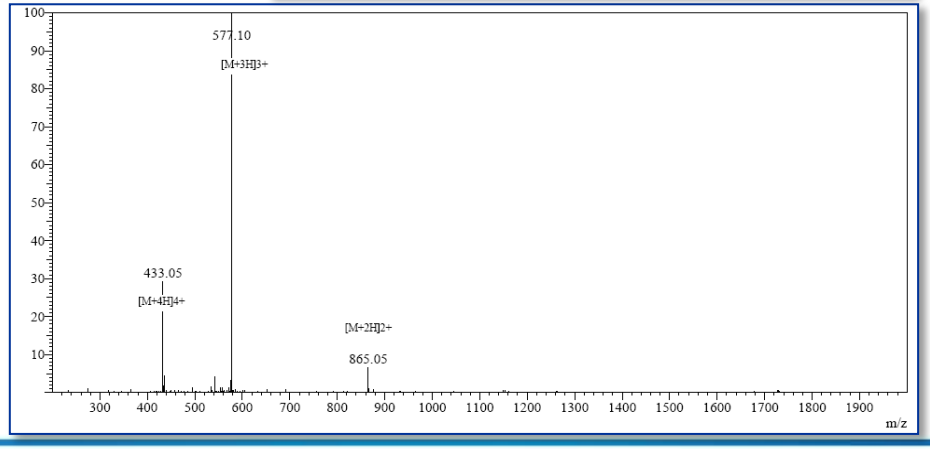
Confirms peptide purity



Peak Table			
Peak#	Retention Time	Area	Area %
1	13.619	139296	1.453
2	13.916	359996	3.756
3	14.054	8707888	90.846
4	14.283	79644	0.831
5	14.400	37342	0.390
6	14.987	261173	2.725
Total		9585339	100.000

MS Trace

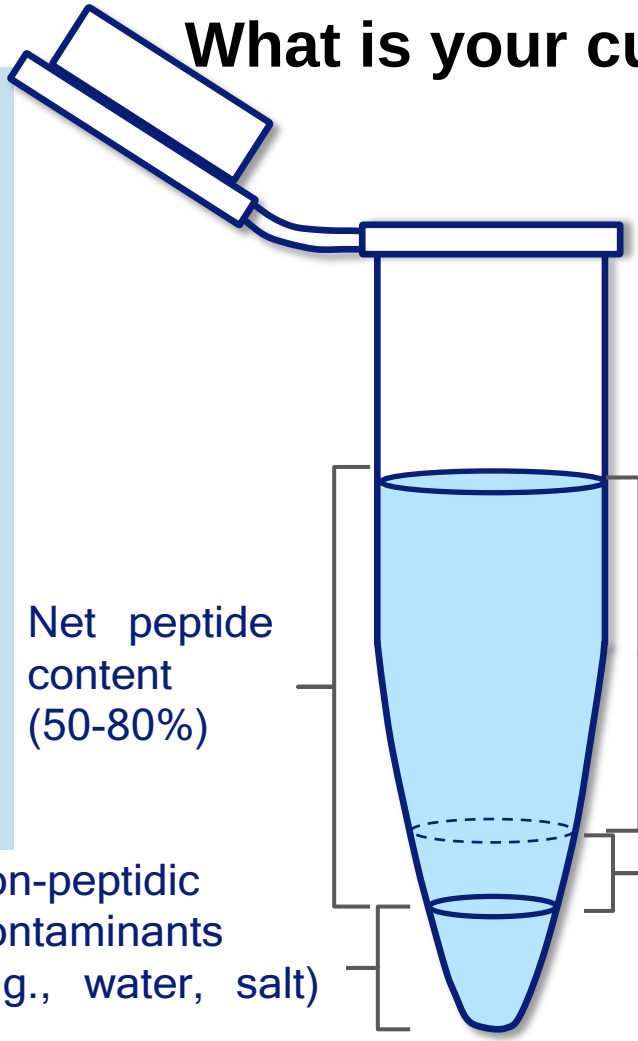
Confirms major ion species by mass



Custom Peptide Composition

What is your custom peptide really made of?

Contamination



Net peptide content (50-80%)

Your peptide sequence (70-95%)

Deletion sequences, truncated sequences

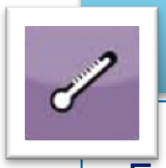
Non-peptidic Contaminants (e.g., water, salt)

A peptide having 95% purity may actually only constitute: (95% x 50%) = 47.5% of the lyophilized powder you receive

Note: Only an Amino Acid Test can tell you the exact concentration of your peptide

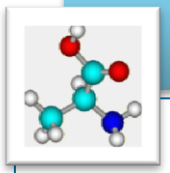
Trifluoroacetic acid (TFA) Counter-ion Contamination

Contamination



Symptoms

- Erratic cell or tissue viability, enzyme assay results
- IR spectroscopy data
- Reduced mass spec sensitivity
- Peptide degradation



Affected Sequences

All sequences, especially those containing positively charged:

- lysine
- histidine
- arginine

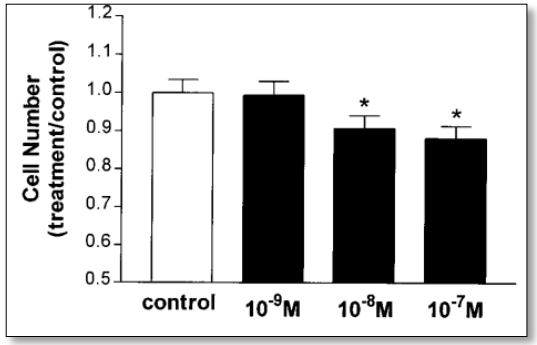
Solution

Protect your assays

↓

TFA removal service

Case Study: TFA effects on cell and tissue culture



Effect of [TFA] on fetal rat osteoblast growth after 24 h. **P* <0.05 indicates data statistical significantly different from control.

Cell Type	Assay Type	[TFA] Effect
Fetal rat osteoblasts	Cell number	↔ (10^{-9} M) ↓ (10^{-7} M - 10^{-8} M)
Canine articular chondrocytes	Cell number	↔ ($4x10^{-9}$ M) ↓ ($4x10^{-8}$ M) ↓ ($4x10^{-7}$ M)
Neonatal mouse calvariae	Thymidine incorporation	↓ (2×10^{-7})

↑ Increase ↓ Decrease ↔ No change

- TFA at concentrations higher than 10^{-9} M can decrease cell and tissue growth

*Figures adapted from Fig. 1 of Cornish J et al. Trifluoroacetate, a contaminant in purified proteins, inhibits proliferation of osteoblasts and chondrocytes. (1999) Am J Physiol Endocrinol Metab 277: E779-E783.

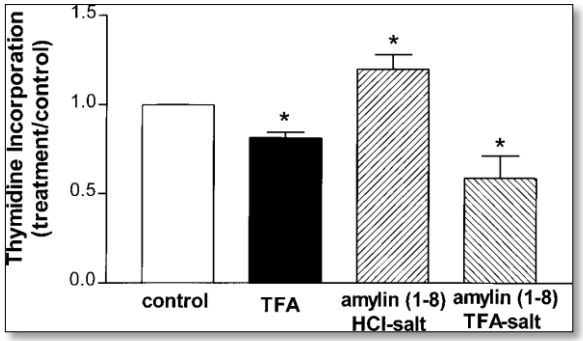
Case Study: TFA effects on cell and tissue culture

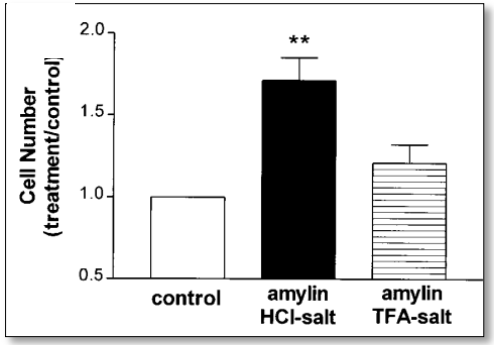
		Cell Type: Fetal Rat Osteoblasts					
		Amylin HCl-salt	Amylin TFA-salt	CGRP HCl-salt	CGRP TFA-salt	Rat calcitonin HCl-salt	Rat calcitonin TFA-salt
Parameter Measured	Cell number	↑	↔	↑↑	↑	↔	↓

		Tissue Type: Neonatal Mouse Calvariae	
		Amylin HCl-salt	Amylin TFA-salt
Parameter Measured	Thymidine incorporation	↑	↓

↑ Increase ↓ Decrease ↔ No change

- Residual TFA in purified peptides can decrease cell and tissue growth



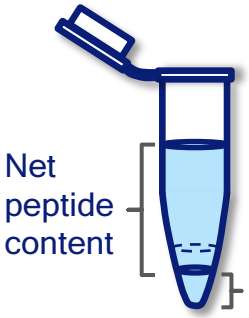


Effect of TFA or HCl purified peptides on cell and tissue growth after 24 h
**P* <0.05 and ***P* <0.003 and indicates data statistical significantly different from control.

*Figures adapted from Fig. 2 and 5. of Cornish J et al. Trifluoroacetate, a contaminant in purified proteins, inhibits proliferation of osteoblasts and chondrocytes. (1999) Am J Physiol Endocrinol Metab 277: E779-E783.

GenScript's guaranteed TFA removal service

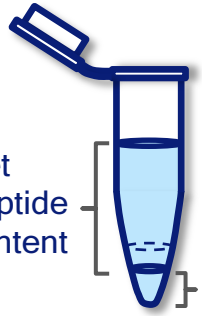
Contamination



Net peptide content

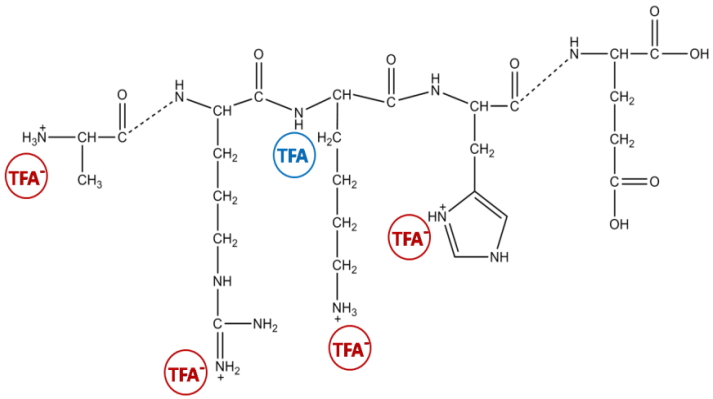
TFA counter-ions, water, salt

Counter-ion exchange

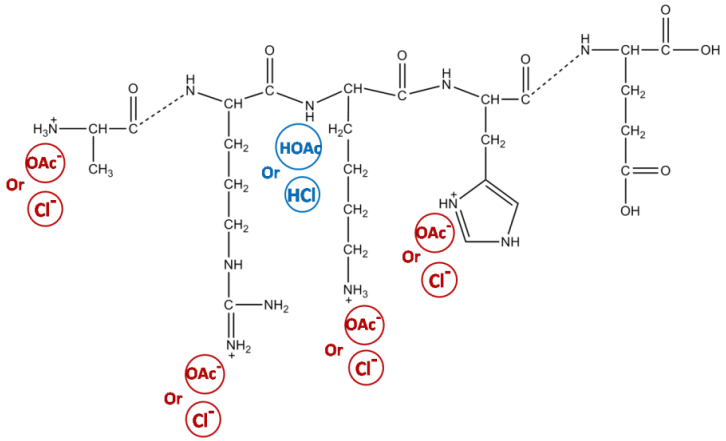


Net peptide content

Acetate or HCl water, salt



10% - 30% TFA



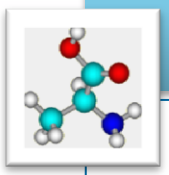
< 0.1% TFA

Peptide Deliquescence

Contamination

Symptoms

- Peptide loses activity over time
- Lyophilized peptide converts from powder to crystal



Affected Sequences

Sequences containing:

- Ser
- Thr
- Lys
- Gly
- Arg

Solutions

Protect your peptide →

- Aliquot peptides
- Store peptides in a desiccator

*Deliquescence: Gradual dissolution by the absorption of moisture from the air

Case Study: Resolving deliquescence

Contamination

A - Lyophilized peptide after treatment with excipients

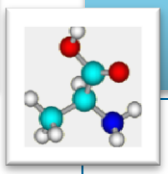
B - Lyophilized peptide without excipient treatment

Peptide Insolubility

Insolubility

Symptoms

Turbidity or precipitation following peptide dissolution



Affected Sequences

Sequences:
>5 AA long, *and* containing >50%:

- Tryptophan
- Isoleucine
- Leucine
- Phenylalanine
- Methionine
- Valine
- Tyrosine

Peptide insolubility solutions

Hydrophobicity

Peptide Protection

- Follow the solubility chart recommendations
- Request solubility testing

Solutions

Sequence Optimization

- Residue substitutions

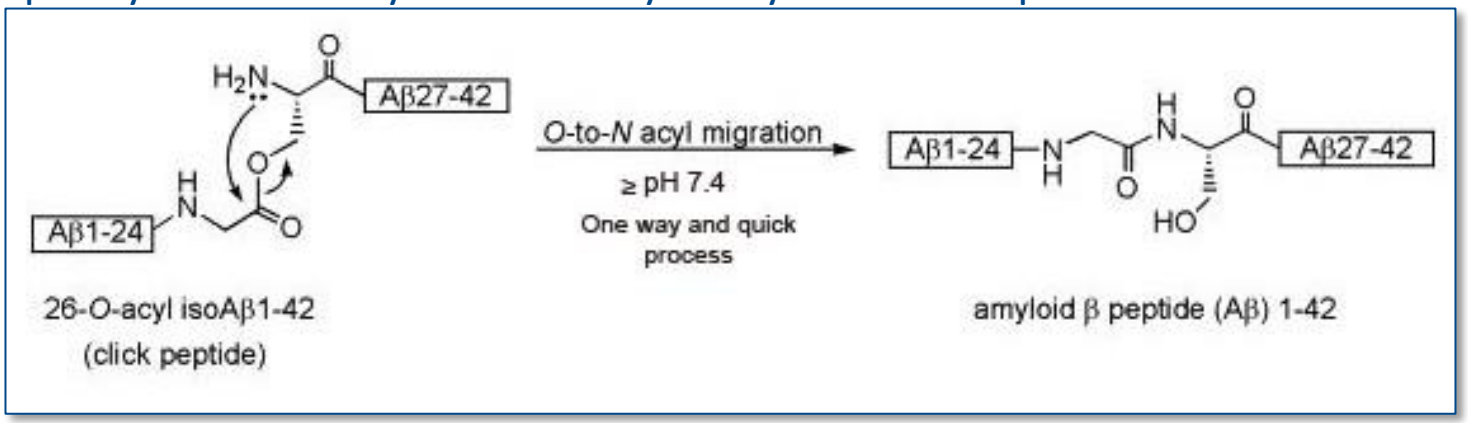
Replace:	With:
Tryptophan	Aspartic acid
Isoleucine	Lysine
Leucine	Arginine
Phenylalanine	Histidine
Methionine	Glutamic Acid
Valine	
Tyrosine	

- Incorporate of O-acyl bonds
- Incorporate hydrophilic linkers (e.g. Lys-Lys-Lys-Lys)
- For libraries introduce a frame shift

Case study: O-acyl bond incorporation

Insolubility

β-amyloid solubility increased by O-acyl bond incorporation



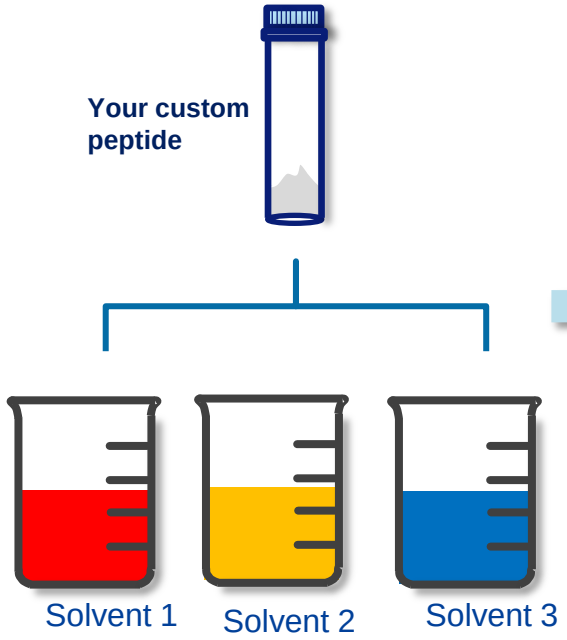
	Click β-amyloid	Native β-amyloid
Solubility in water	15 mg/ml	0.14 mg/ml

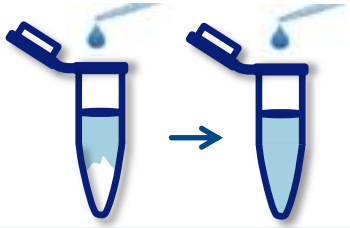
Kiso et al. (2008) Click Peptides: Design and Applications

*GenScript's Click peptide service introduces a an o-acyl bond into the peptide sequence can greatly increase solubility

Solubility Testing Service

Insolubility





Example Solubility report results

Solvent	pH Value	Dissolution Achieved?	Peptide Concentration
Aqueous solution		Undissolved	N.A.
		Dissolved	mg/mL
		N.A.	N.A.
Buffer	pH 5	Dissolved	mg/mL
	pH 7	N.A.	N.A.
	pH 10	N.A.	N.A.
Organic solvent		Undissolved	N.A.
		Dissolved	mg/mL

Peptide Instability: Design Considerations

Instability

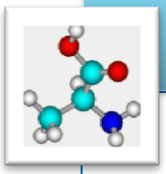
	Susceptible amino acids	Solutions
Cyclization	▪ Asp-Gly, ▪ N-terminal Glu	▪ Substitute Asp with other amino acids ▪ Acetylate N-terminal Glu
Secondary structure formation	Multiple Glu, Ile, Leu, Phe, Thr, Tyr, Val	▪ Asp for Glu ▪ Ser for Thr ▪ Pro or Gly every third residue
Oxidation	▪ Cys ▪ Met	▪ Cysteine with Serine ▪ Methionine with Norleucine
Deliquescence	▪ Ser ▪ Thr ▪ Lys ▪ Gly ▪ Arg	Substitute non-essential charged residues with uncharged residues
Hydrolysis	▪ Asp-Gly ▪ Asp-Pro ▪ Asp-Ser ▪ Asp-Gly	Substitute Asp with other amino acids
Deletion sequences	Multiple adjacent Ser	Substitute Ser with other amino acids

Peptide Oxidation

Instability

Symptoms

- Peptide loses activity over time
- Peptide color changes from white to yellow, tan or brown



Affected Sequences

Sequences containing:

- Cysteine
- Tryptophan
- Methionine

Peptide Oxidation

Instability

Solutions

Design Optimization

Replace:

- Cysteine with Serine
- Methionine with Norleucine

Peptide Protection

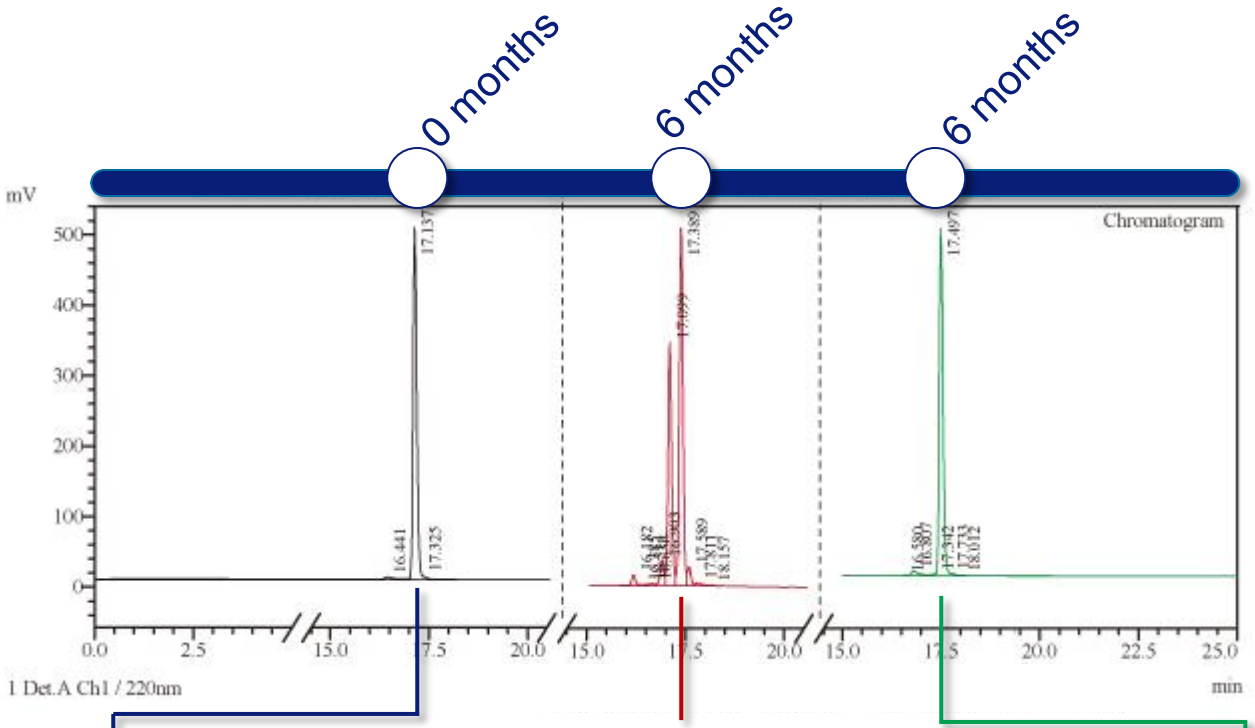
- Aliquot peptides
- Flush peptides with argon gas and store in a tightly sealed container

Assay Protection

- Add reductants: DTT, TCEP, β-mercaptoethanol,
- Flush buffers with argon gas
- Reconstitute buffers and peptide with argon-flushed water in an anaerobic chamber
- Perform assays in anaerobic chamber

Case Study: Effects of Oxidation

Instability



Peptide after synthesis and purification

Peptide *without* ArgonShield™ protection after 6 months of storage

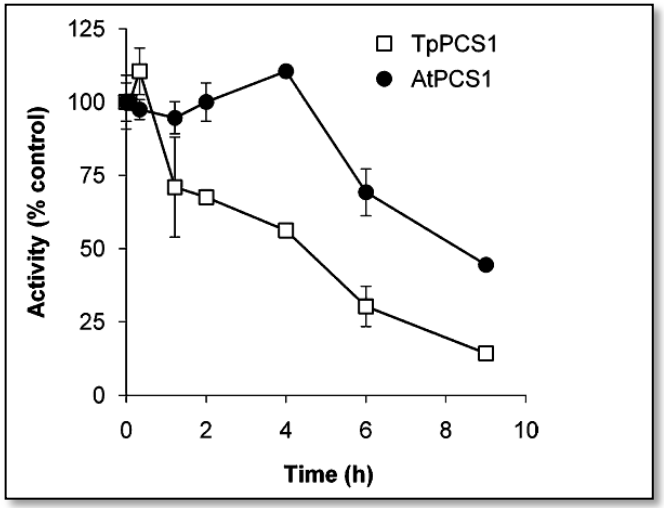
(smaller peaks denote new oxidized peptide species)

Peptides with ArgonShield™ protection after 6 months of storage

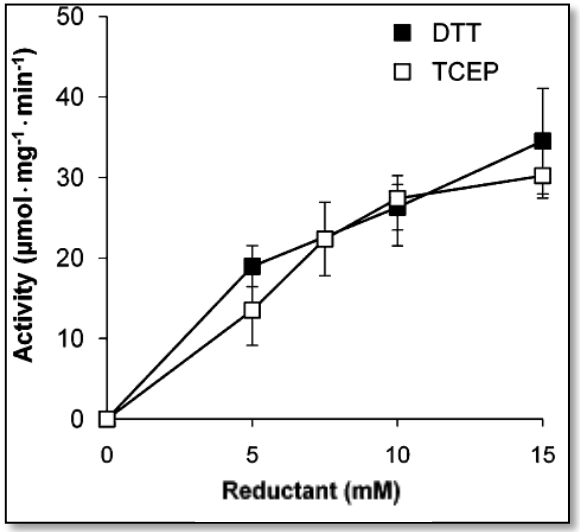
*Argonshield™ Technology protects peptides by applying an argon gas headspace and airtight seal

Case study: Reducing/preserving oxidative residues

Instability



*Oxidation sensitive dipeptydyl transpeptidase enzyme activity assayed after experimental preparation and removal from an anaerobic chamber



*Oxidation sensitive dipeptydyl transpeptidase enzyme activity assayed with increasing levels of reductants

*Adapted from Fig. 4 and 5 of Gupton-Campolongo T et al. (2013) J. Phycol. 49:32-40

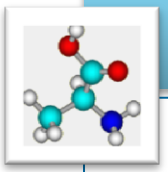
Peptide Color Change

Instability

Symptoms

- Erratic colorimetric assay data
- Lyophilized peptide is tan, yellow or brown

Affected Sequences



Sequences containing:

- Trp
- Tyr
- Cys
- Glu
- Asn

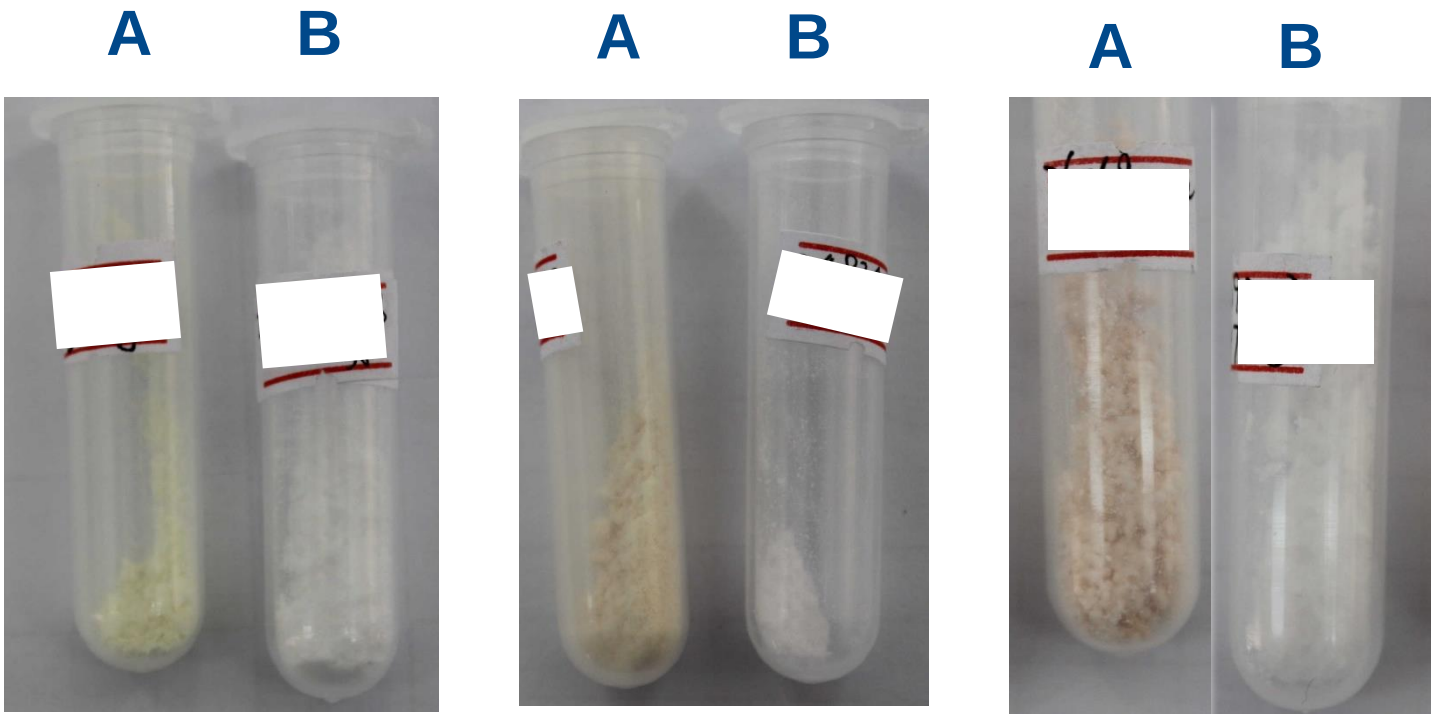
Solutions

Color removal service

*Lyophilized peptides can change color due to oxidation, or contamination by colored protective groups

Case Study: Removing Peptide Color

Instability



A - Lyophilized peptide after synthesis

B – Lyophilized peptide after proprietary de-colorization treatment

Resources: Stability Guidelines Chart

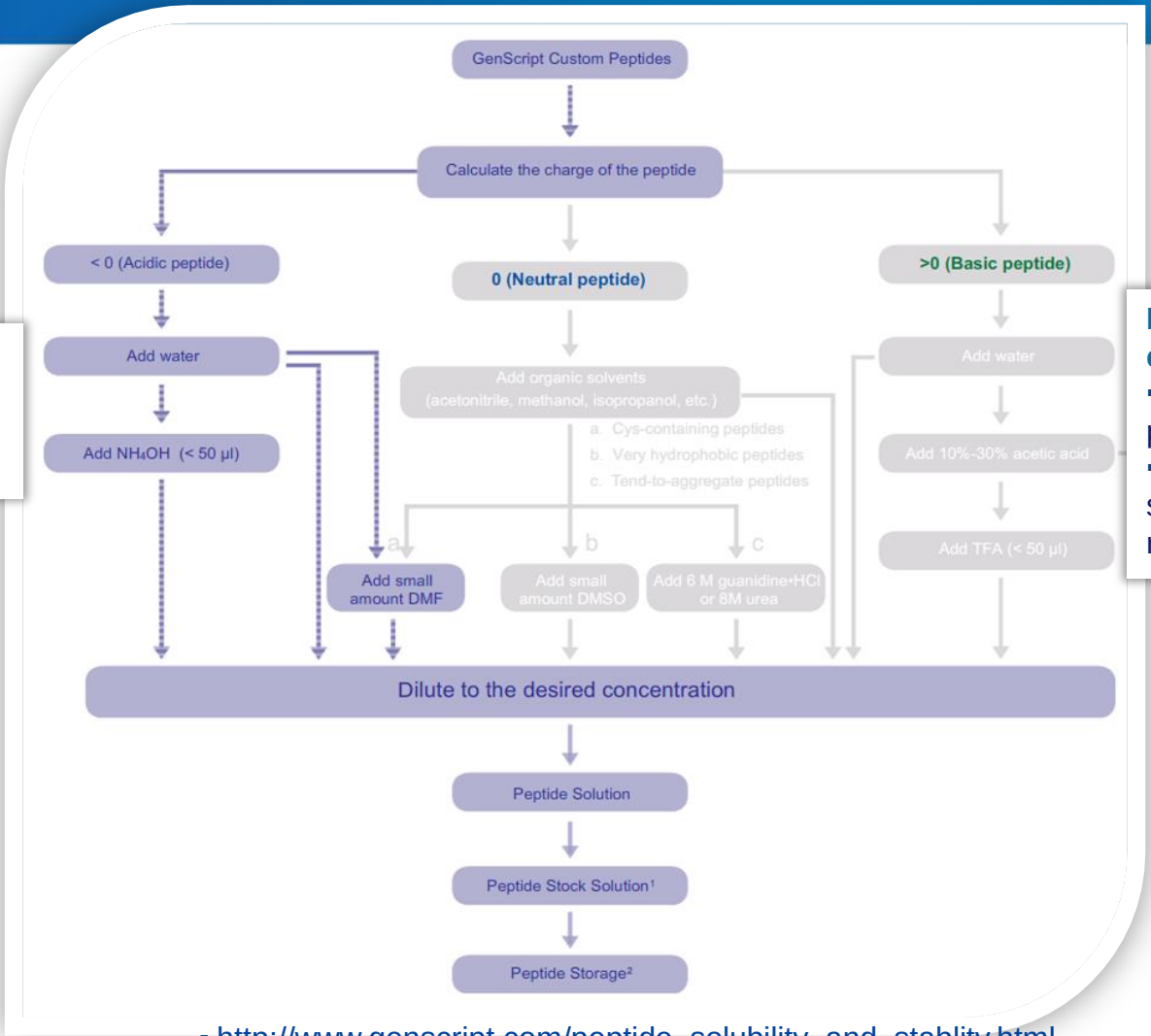
Do's and Dont's of Peptide Handling

For ALL peptides	•DO aliquot lyophilized peptide according to daily experimental needs (Try GenScript's free aliquoting service) •DO avoid repeated freeze-thaw cycles •DON'T store peptides long-term in solution •DON'T repeatedly open the stock peptide vial
For peptides containing Cys, Met, or Trp residues	•DO limit peptide exposure to air •DO purge assay buffers with argon or nitrogen gas •DO store peptides in tightly capped vials
For peptides containing Asp, Glu, Lys, Arg, or His	•DO limit peptide exposure to air •DO store lyophilized peptides in a desiccator •DO store peptides in tightly capped vials
For peptides that must be stored in solution	•DO avoid repeated freeze-thaw cycles •DO aliquot your peptide solution according to daily experimental needs •DO use sterile buffers to dissolve your peptide •DO filter your peptide using a 0.2 μm filter to remove bacterial contamination

http://www.genscript.com/peptide_storage_and_handling.html

Resources: Design Tools

Peptide MW calculator
- Calculates MW considering a selection of over **60** modifications



Peptide Property calculator
- Calculates isoelectric point
- Generates automatic solubility recommendations

- http://www.genscript.com/peptide_solubility_and_stablity.html
- https://www.genscript.com/ssl-bin/peptide_mw
- https://www.genscript.com/ssl-bin/site2/peptide_calculation.cgi

Summary

Categories	Solutions	Specialized Services
Contamination	▪ Amino acid substitutions ▪ Storage in desiccator	▪ TFA counter-ion removal ▪ Moisture removal
Hydrophobicity	▪ Amino acid substitutions ▪ O-acyl bond incorporation ▪ Hydrophilic linker incorporation ▪ Frame shifting	▪ O-acyl bond incorporation ▪ Free Solubility testing
Instability	▪ Amino acid substitutions ▪ O-acyl bond incorporation ▪ Aliquoting ▪ Application of argon gas headspace ▪ Use of reductants in assay ▪ Perform assay in anaerobic chamber	▪ Free Aliquoting ▪ Argonsheild™ ▪ De-colorization service

GenScript's Specialized Services List

Service	Price	Turnaround Time (in addition to peptide synthesis)	Deliverables
Amino acid analysis	Typically $100 + 5 mg additional peptide, sequence dependent	1 week	▪ Amino acid analysis test report
TFA Removal	Typically $70 + 5 mg additional peptide, sequence dependent	2 weeks	▪ Lyophilized peptide ▪ TFA content test report
Click peptide service	Sequence dependent	No additional time	▪ Lyophilized peptide
Solubility testing	Free	No additional time	▪ Lyophilized peptide ▪ Solubility test report
ArgonSheild™	Free	No additional time	▪ Lyophilized peptide in argon flushed, sealed vials
De-colorization treatment	Project dependent	1 week	▪ White Lyophilized peptide (by visual assessment)
Moisture removal	Project dependent	1 week	▪ Lyophilized peptide (by visual assessment)
Aliquoting	Free for first 5 vials, then $2.00 per vial	No additional time	▪ Lyophilized peptide aliquoted into sepcified # of vials

Thank you for your participation

We wish you all success in your Research

Email me: Tiffany.Campolongo@GenScript.com

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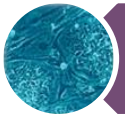
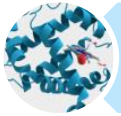
May 21, 2014/ 11:00 am EST
Gene variant libraries: design, construction, and research applications - *Rachel Speer, Ph.D.*

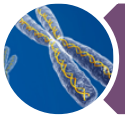
May 28, 2014/ 1:00 pm EST
Protein or peptide antigen: choosing the optimal immunogen for antibody production - *Jessica Kaplunov, Ph.D.*



June 5, 2014/ 2:00 pm EST
Stem cell culture: choosing optimal conditions for expansion and differentiation - *Matthew Riolo, Ph.D.*

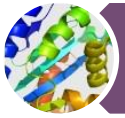


June 11, 2014/ 1:00 pm EST
Recombinant protein expression & purification: challenges and solutions - *Liyan Pang, Ph.D.*



June 18, 2014/ 2:00 pm EST
Can CRISPR/Cas9 off-target genomic editing be avoided? Ways to improve target specificity - *Maxine Chen, Ph.D*

June 25, 2014/ 2:00 pm EST
Building a Synthetic Eukaryotic Genome – Sc2.0 - *Leslie Mitchell, Ph.D., NYU Langone Medical Center*



On Demand (Originally aired May 8, 2014)
Optimizing conditions for recombinant soluble protein production in *E. coli*, - *Keshav Vasanthavada*