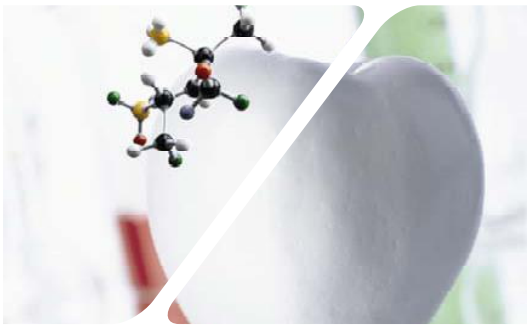
2006

MOVING FORWARD

WITH QUALITY CUSTOM SYNTHESIS



BIOSYNTHESIS INC
SYNTHESIS THROUGH INGENUITY

A TRADITION OF EXCELLENCE

QUALITY CUSTOM SYNTHESIS

Since 1984

DESIGNED TO FULFILL THE NEEDS OF TODAY'S DRUG DISCOVERY THROUGH LIFE SCIENCE RESEARCH

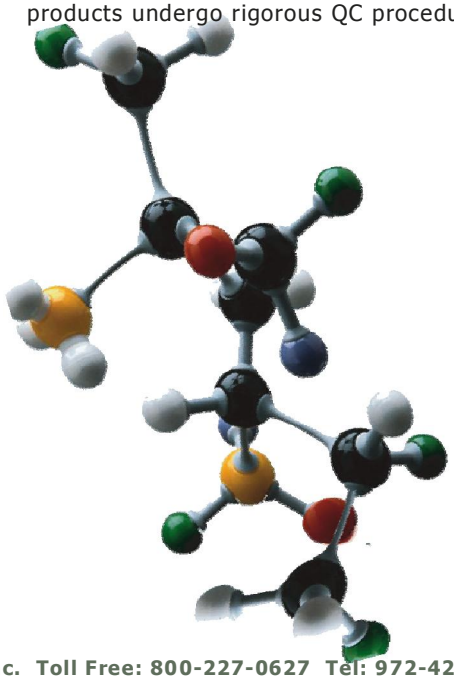
Bio-Synthesis, Inc., established in 1984, is a chemistry-based company specializing in the design and synthesis of peptides, small molecules and reagents for both research and bulk pharmaceutical trials. Using state-of-the-art technology in our well-equipped laboratories, coupled with a staff possessing decades of experience in synthesis chemistry, enables us to deliver high quality **PEPTIDES**, **DNA**, **ANTIBODIES**, **CONTRACT SYNTHESIS**, **PROTEOMIC ANALYTICAL SERVICES**, and **MOLECULAR DIAGNOSTIC REAGENTS** with speed and flexibility. Since our inception in 1984, we have assisted researchers worldwide in the design and synthesis of custom molecules with a wide variety of requirements from micro-scale high throughput to macro-scale production. Our assurance to our customers is based on a simple philosophy: we measure our success on the success of our clients. In a customer-driven world, there is no other standard. Our clients' success constitutes an integral part of our business model and their needs are the first priority of **BSI**. Below you may find some of the companies we are proud to work with. The majority of the projects we have performed for them have been highly sophisticated, requiring substantial chemistry skills, innovative input and dedicated personnel. Whatever the size of the company or project, we've always been committed to long-standing, mutually beneficial and trustworthy relationships.

- Merck . Pfizer . SmithKline Beecham . Lawrence Berkeley

For more information visit www.biosyn.com

COMPREHENSIVE CUSTOM SYNTHESIS AND SERVICES

At **BSI**, we recognize that providing faster delivery of products is critical to expediting research. **We are committed to our customers' timely needs** while maintaining product quality and integrity. All of our products undergo rigorous QC procedures with full analytical packages supplied with every product.



- PEPTIDE SYNTHESIS
- BIOPNA SYNTHESIS
- ANTIBODY PRODUCTION
- CONTRACT ORGANIC SYNTHESIS
- DNA SYNTHESIS
- ANALYTICAL SERVICES
- MOLECULAR DIAGNOSTIC REAGENTS

Bio-Synthesis, Inc. Toll Free: 800-227-0627 Tel: 972-420-8505 Fax: 972-420-0442 Web: www.biosyn.com Email: biosyn@biosyn.com

CUSTOM PEPTIDE SYNTHESIS

An Overview

OUR UNIQUE CONTRIBUTION

- Extensive History and Experience
- Solid and Solution Phase Chemistries
- Optimized Fmoc and Boc Methodologies
- Small Scale High Throughput to Bulk Scale Contract Synthesis
- Small to Long Peptides (2-120 aa)
- Multi-Purity Levels with a Wide Variety of Modifications
- FREE Peptide Design and Consultation
- State-of-the-art Facilities with Internal Engineers, 24/7 Maintenance Support and Technical Staff to ensure COMPLETE QUALITY CONTROL

SYNTHESIS PLATFORMS and CAPABILITIES

BSI is unique in that we have our own internal engineers, maintenance support, and technical staff available 24 hours a day for our state-of-the-art instrumentations and laboratories. We are always ready to meet large volume, micro or macro scale synthesis with a wide variety of custom modifications according to research needs.

Our peptide synthesis is fully automated from small to large scale synthesis following GLP and cGMP guidelines. All peptides are synthesized using either solid or liquid phase chemistry, and if necessary, the application of Boc chemistry. Fmoc based chemistry is available using either diisopropylcarbodiimide or HBTU for activation of individual amino acids. Complete peptides are deprotected and cleaved from the solid support using trifluoroacetic acid containing the appropriate scavengers (thiol based scavengers). The extracted peptides are precipitated into MTBG, then centrifuged at 5000 rmp to form a pellet, which is washed several times with water. At this point peptides are purified by HPLC using reverse phase C4,C8, and C18 columns. Alternate purification methods are ion exchange and gel filtration chromatography. Final products are confirmed with MALDI-TOF mass spectrometry, amino acid analysis or peptide sequencing, if necessary.

QUALITY CONTROL/ASSURANCE

After synthesis is complete, upon request, peptides are purified by reverse phase HPLC on Dionex, Waters, Pharmacia instrumentations and others using C18,C8 or C4 Vydac silica columns eluted with trifluoroacetic acid-based/ acetonitrile gradients. A linear gradient of 10% B to 80% B in 45 minutes at a flow rate of 20 ml a minute (solvent A:0.05% TFA in water: solvent B:0.05% TFA in acetonitrile) is used. The major peaks are analyzed by Time-of-Flight Mass spectrometry on an Applied Biosystem Voyager DE or DERP Mass Spectrometer. The fractions with the correct mass are pooled and then lyophilized. In addition, amino acid analysis and/or peptide sequencing is available upon request. Amino acid analysis is performed using Water's Breeze Systems.

QUALITY BEYOND YOUR EXPECTATIONS

CUSTOM PEPTIDE SYNTHESIS

Continued...

STANDARD PEPTIDE SYNTHESIS

Scale	1.0mg to gram
Length	8 - 45 aa
Purity	Desalted, >75%, >85%, >95%

Includes: Mass Spec Analysis, HPLC

Optional Services: Amino Acid Analysis and Sequencing

Contract volume and bulk scale synthesis are available
Call **800-227-0627**or **biosyn@biosyn.com**

LARGE (LONG) PEPTIDE SYNTHESIS

Most commercial proteins are made by recombinant methods, whereby gene coding for particular peptide sequences are inserted into a host organism for bulk synthesis. While endotoxin contamination can be problematic for recombinant methods, chemical synthesis offers much cleaner alternatives.

BSI has decades of experience and has synthesized >500,000 custom peptides, many of them long peptides ranging from 50 to 120 residues with QUALITY and SPEED. Write to us at **biosyn@biosyn.com** for a price quotation.

FLUORESCENCE / STABLE ISOTOPE LABELING

Fluorescence Dye	• Texas Red • Fluorescein • Tetramethylrhodamine	• Bodipy • Aminocoumarin • And much more...
Stable Isotope	• C13	• N15

Standard quantity: 10 mgs. Large quantities also available

MODIFICATION DEVELOPMENT

Regiospecific Modification/ Structure Modification	• **BIOPNA** Peptide Nucleic Acid • Acetylation • **BIOCONJUGATION** • Cyclization (internal/external) • Sulfonation (Y) • Matrix Immobilization • Phosphorylation (S,T,Y) • Alkylation • Glycopeptides • Lipopeptides • Tripalmitoyl-S-Glyceryl Cysteine (PAM3 Cys-OH)	• Succinylation • Farnesylated Peptide • Amidation • Fluorescent Tags • Protected Fragments • Biotinylation • Myristylation • Modified Amino Acids • Unnatural Amino Acids • Multiple Antigen Peptide • And much more...

Standard quantity: 10 mgs. Large quantities also available

BIOSCREEN PEPTIDES

Custom Peptide Libraries

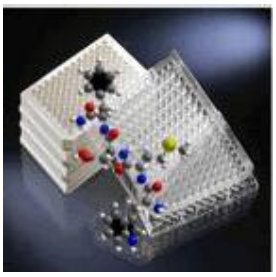
CUSTOM PEPTIDE

Large numbers of extremely affordable custom peptides have never been easier....

Using state-of-the-art technology in our facilities, coupled with in-house engineers and a 24/7 synthesis support team enables **Bio-Synthesis** to offer parallel synthesis of small quantities of peptides with the fastest, most efficient high throughput. As a result, our **BIOSCREEN** peptide array program offers 96 different peptides, unbound, in a 96-well format. Each plate is individually tested for accuracy and can be used for epitope mapping, protein characterization, high throughput screening and much more. With this synthesis design, large numbers of peptides are delivered rapidly and affordably in a short time, adding great value to our customers' budgets.

FEATURES

Quantity	0.5-2.0 mgs
Purity	Average 50-90%
Length	8-15 Amino Acids
Modifications	N-terminus modification and non-standard amino acids available, upon request
Order Size	96 peptides
QC	MALDI-TOF mass spectrometry on all peptides
Packaging	96 lyophilized crude peptides in a 96-well format
Includes	Electronic QC data
Delivery	7-10 business days



OTHER BIOSCREEN NOTES AND INFORMATION

MODIFICATIONS

- **N-TERMINAL BIOTIN**
- **N-TERMINAL FLUORESCEIN**
- **D-AMINO ACIDS**
- **NON-STANDARD RESIDUES**
- **C-TERMINAL ACID OR AMIDE**

APPLICATIONS

- Defining minimum protein-protein interaction domain: epitope mapping
- Key residue identification: identifies the residue(s) involved in binding
- Interactions between protein and other molecules, protein-DNA, protein - polysaccharide, and protein - cell, protein - metal interactions
- General drug screening: interactions to determine drug leads
- Protein conformation probes: evaluation of binding for multiple binding domains

TECHNICAL NOTES

BIOSCREEN peptide arrays are designed for high throughput screening, and peptide purities may vary from 50-95%. We routinely run tests of our synthesis platform to ensure quality of the product. The average crude purity of **BIOSCREEN** peptide arrays are:

10 amino acid residues: ~85%

15 amino acid residues: ~75%

20 amino acid residues: ~65%

BSI recommends peptide lengths of 15 amino acids or less. Because the yield may be low, hydrophobic peptides are discouraged.

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PEPTIDE SYNTHESIS

Technical Notes...

OPTIONS FOR PEPTIDES THAT ARE DIFFICULT TO SYNTHESIZE

Peptides are complex molecules and each sequence is unique with regard to its chemical and physical properties. While some peptides are difficult to synthesize, others are relatively straight forward, but difficult to purify. Another common problem with many peptides is insolubility in aqueous solution. To aid you in the design of your peptide sequence, we offer these general guidelines.

1. Shorten the sequence

Generally speaking, as peptide length increases, the yield and purity of the crude product becomes lower. Most peptides of 15 residues or fewer can be synthesized without major difficulty, but with peptides longer than 20 residues, yields generally decrease.

2. Decrease the number of hydrophobic residues

Peptides with a predominance of hydrophobic residues, especially in the region of 7-12 amino acids from the C-terminus, often have assembly problems. This is thought to cause ß-sheet formation between peptide chains during synthesis, which causes incomplete coupling. In these cases, replacing one or more residues with a more polar residue, or by adding a Gly or Pro residue to help break up the regular peptide structures may help.

3. Minimize difficult residues

Peptides with multiple cysteine, methionine, arginine and tryptophan are often difficult to synthesize. Serine can be used as a non-oxidizing replacement for cysteine, and norleucine can be used as a methionine replacement. Lysine can be used in place of arginine while tyrosine or phenylalanine or other hydrophobic residues such as leucine are sometimes adequate replacements for tryptophan.

OPTIONS TO IMPROVE SOLUBILITY

1. Change N or C terminus

For acidic peptides, those having an overall negative charge at pH7, we recommend a peptide format ***Acetyl-peptide-COOH*** (acetyl group at amino terminus and free acid at carboxy terminus) to maximize the negative charge. For basic peptides (i.e. peptide has an overall positive charge pH7), we recommend a peptide format of ***H-peptide-amide*** (free N terminal amino group, and amide C-terminus) to maximize the positive charge.

2. Shorten or lengthen sequence

Some sequences contain a large number of hydrophobic residues such as Trp, Phe, Val, Ile, Leu, Met, Tyr and Ala. Generally we see solubility problems in peptides where >50% of the residues are these hydrophobic amino acids. In order to increase the polarity of the peptide, it may be useful to lengthen the sequence, provided the added amino acids increase peptide polarity. Alternatively, the sequence may be shortened to eliminate hydrophobic residues and, hence, increase peptide polarity. The more polar the peptide, the more likely it is to be soluble in aqueous buffers.

3. Add solubilizing residues

For some peptides, it is possible to arbitrarily add a set of polar residues to improve solubility. We recommend for acidic peptides to add Glu-Glu to the N or C terminus and for basic peptides to add Lys-Lys to the N or C terminus. If a charged group cannot be tolerated, we recommend the addition of Ser-Gly-Ser to the N or C-terminus. Obviously there are cases where the N and C termini cannot be altered, and this approach would not be applicable.

4. Alter sequence by substituting one or more residues

Peptide solubility may be improved by changing some residues within the sequence. Often, a single replacement (e.g., replacing alanine with glycine) can dramatically improve solubility but remains relatively conservative.

5. Alter the sequence by selecting a different frame or overlapping peptides

If a number of sequential or overlapping peptides of set length forming a sequence are to be made, a change in the starting point of each peptide may make a difference. This creates a better balance between hydrophobic and hydrophilic residues in individual peptides, or by separating difficult residues into different peptides (e.g. two cysteines into separate peptides instead of together in one peptide).

RECONSITUTION OF PEPTIDE

Peptides have a wide range of properties which the following guidelines may not address. If you require more information concerning a specific peptide, please call our technical staff at **800-227-0627**. Use only a small amount of peptide to test for solubility. *Only* when the peptide has been fully dissolved should the buffer salts be added and the solution diluted to its final concentration.

NOTE: Always add the peptide into a stirring solution of appropriate solvents, not the other way around

1. The main problem associated with the dissolution of a peptide is the formation of aggregated secondary structures. Although secondary structures are more pronounced with hydrophobic peptides, they are liable to occur with all except the shortest of peptides, regardless of polarity. Therefore, the first rule is to try to dissolve the peptide in sterile, distilled or deionized (and, if possible, oxygen-free) water.

A. Bacterial degradation can be a problem with peptide solutions. To avoid this, peptides should be dissolve in sterile water or have the peptide solution sterile filtered using a 0.45 cm micron or 0.2 micron filter.

B. Peptides containing cysteine, methionine, or tryptophan are particularly susceptible to oxidation and should be dissolved in oxygen free water. This can be prepared by degassing under reduced pressure and replacing with an inert gas such as nitrogen, helium or argon.

2. If the peptide is insoluble in pure water, sonication may help break up any particles and increase the rate of dissolution.

CAUTION: *Sonication can cause warming of the solution and degradation of the peptide.*

3. If the peptide contains many basic amino acids, use an aqueous acetic acid (1 to 10%) solution, with or without sonication. For very hydrophobic peptides, use a 50% aqueous acetic acid.

PEPTIDE SYNTHESIS

Technical Notes Continued

4. If the peptide has many acidic amino acids, use an aqueous ammonia (1 to 10%) solution, or a volatile basic buffer (up to pH8) such as N-ethylmorpholine acetate or bicarbonate with or without sonication. The pH may have to be adjusted before chromatography.

5. Propanol and acetonitrile can dissolve some medium-size peptides. If the peptide is to be injected onto a column, the amount of organic solvent, especially propanol, must be kept small, or retention time will be greatly affected.

6. If the peptide is highly hydrophobic with aromatic or hydrocarbon-like side chains, such as Val, Leu, Ile, Met, Phe, Tyr, Ala, or if the peptide is neutral, use a chaotropic agent such as DMF or DMSO.

A. High concentrations of chaotropic salts help to dissolve the peptide by breaking up the secondary structures.

B. Chaotropic agents are suitable for preparing solutions for analysis, but may interfere with a biological system used for the study of the peptide.

C. The best agent is DMF (up to 30%), added drop by drop, until the peptide dissolves.

D. On reverse-phase chromatography, the DMF will elute with the buffer front. The UV absorbance can be very high, depending on how much is injected. Most peptides are retained longer than the few minutes it takes for DMF to elute. If the peptide is very small and elutes early, the introduction of the organic solvent component of the mobile phase should be delayed following injection.

PEPTIDE STORAGE

LYOPHILIZED FORM: All products marked 'keep cool and dry' should be stored frozen, preferably at -20°C. Most peptides, when stored below –70°C, will remain stable for several years. This also applies to many amino acid derivatives, lipids, enzymes and other proteins. The only exception to this are immobilized proteins, which should not be frozen.

When using a frozen product, the bottle or vial should be allowed to warm to room temperature in a desiccator containing fresh desiccant before opening. This process can take an hour or more from -20°C, depending on the pack size.

Failure to do this can cause condensation to form on the product when the bottle or vial is opened and will greatly reduce the stability of the material. Once opened, the required quantity should be weighed out and the vial or bottle re-sealed immediately to prevent absorption of water. This is a particular problem with some hydrophobic peptides.

SOLUTION FORM: Peptides in solution are much less stable than lyophilized peptides. For best results, follow these guidelines:

1. It is recommended that the stock solution be aliquoted upon arrival to prevent degradation caused by repeated thawing and freezing. Thaw only what is needed and discard any unused portion.

2. Sterile filter before storing to prevent bacterial contamination.

3. Maintain peptides in an oxygen-free environment as peptides containing cysteine, methionine, tryptophan, glutamine, and asparagines are susceptible to oxidation and have limited shelf life.

4. For maximum stability, store at -20°C, in a sterile, pH 5 to 7 buffered solution.

A COMPREHENSIVE SOLUTION

ANTIBODY PRODUCTION

Overview

FROM DESIGN TO SERUM PRODUCTION
A ONE STOP SOLUTION...DESIGNED JUST FOR YOU

For over 20 years, **Bio-Synthesis** has provided custom antibody production services around the world. We offer comprehensive services for polyclonal antibody production with large selections of host animals. Each of our facilities is operated under USDA license and holds an NIH Animal Welfare Assurance from the Office of Laboratory Animal Welfare.

From antigen selection to antibody purification, **BSI** has optimized each step of the antibody production process to provide the best antibodies available.

COMPREHENSIVE SERVICES

Our comprehensive services for polyclonal antibody production include:

- ANTIGEN PREDICTION
- PEPTIDE SYNTHESIS
- ANTIGEN PREPARATION
- ANTIBODY PRODUCTION
- ELISA SCREENING
- ANTIBODY PURIFICATION

SERVICE OPTIONS

We offer several custom antibody service options:

- THE COMPREHENSIVE SOLUTION PACKAGE
- THE ANTIBODY IgG PACKAGE
- EPITOPE AFFINITY PACKAGE
- CUSTOMER SUPPLIED PROTEIN PACKAGE

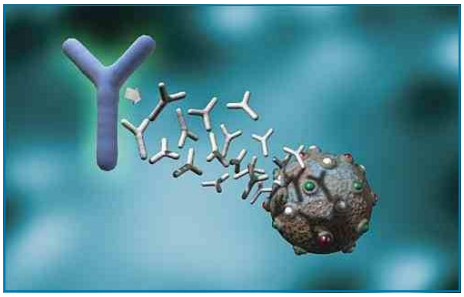
visit us at www.biosyn.com

TOTAL SUPPORT

ANTIGEN PREDICTION
- FREE consultation on peptide selection
- Expert advise on conjugation strategy

PEPTIDE SYNTHESIS
- Multi-purity level
- Varieties of modifications
- QC by HPLC, Mass Spec, AAA (optional)

CARRIER CONJUGATION
- Optimized conjugation methodologies
- Range of carrier protein available
- Analysis of peptide conjugate



IMMUNIZATION
- Proven immunization protocol
- Large selection of host animals

SCREENING
- Anti-peptide activity monitored entirely by ELISA
- Direct communication with project manager

PURIFICATION
-Antibody IgG
-Epitope specific purification
-Fluorochrome conjugation
- and more...

Bio-Synthesis, Inc. Toll Free: 800-227-0627 Tel: 972-420-8505 Fax: 972-420-0442 Web: www.biosyn.com Email: biosyn@biosyn.com

RABBIT ANTIBODY PRODUCTION PACKAGES

ECONOMY CLASS	FIRST CLASS	SUPER CLASS
• Synthesis at >70% pure • Up to 20 residues	• Synthesis at >80% pure • Up to 20 residues	• Synthesis at >90% pure • Up to 20 residues • **IgG purification of 5 mls**

Features:

- Mass spectral and HPLC tracing of peptide
- Conjugation of peptide to carrier protein
- Unconjugated peptide shipped to researcher
- Immunization of 2 rabbits
- ELISA Testing
- 100 - 150 ml crude serum
- 10-14 weeks delivery

SHEEP & GOAT ANTIBODY PRODUCTION PACKAGES

Services:	Features:
• **ECONOMY CLASS** • **FIRST CLASS** • **SUPER CLASS**	• Mass spectral and HPLC tracing of peptide • Conjugation of peptide to carrier protein • Unconjugated peptide shipped to researcher • Immunization of 2 animals • ELISA Testing • 500 - 1000 ml crude serum • 10-14 weeks delivery

CHICKEN ANTIBODY PRODUCTION PACKAGES

Services:	Features:
• **ECONOMY CLASS** • **FIRST CLASS** • **SUPER CLASS**	• Mass spectral and HPLC tracing of peptide • Conjugation of peptide to carrier protein • Unconjugated peptide shipped to researcher • Immunization of 2 animals • ELISA Testing • 50 - 100 ml crude serum • 10-14 weeks delivery

RAT ANTIBODY PRODUCTION PACKAGES

Services	Features:
• **ECONOMY CLASS** • **FIRST CLASS** • **SUPER CLASS**	• Mass spectral and HPLC tracing of peptide • Conjugation of peptide to carrier protein • Unconjugated peptide shipped to researcher (if available) • Immunization of 2 animals • ELISA Testing • 15 - 25 ml crude serum • 10 - 14 weeks delivery

ANTIBODY PRODUCTION

Continued...

MULTIPLE ANTIGEN (MAP) STRATEGY

MULTIPLE ANTIGENIC PEPTIDES (MAP)

MAP is an another method for producing high titer anti-peptide antibodies[1,2] . This new concept was introduced to avoid chemically undefined entities of an antigen-carrier system. The MAP system utilizes a peptidyl core of three or seven radially branched lysine cores to form a backbone for which the antigen sequences of interest can be built in parallel, using standard solid-phase chemistry. The heptalysine core yields the MAP bearing four or eight copies of the peptide epitope with a resulting three dimensional molecule, which has a high molar ratio of peptide antigen to core molecule. Therefore, it does not require the use of a carrier protein to induce an antibody response. These high molar ratios and dense packing of multiple copies of the antigenic epitope in a MAP have been shown to produce a strong immunogenic response [1,2].

It should be noted that there are synthesis concerns when making a MAP complex. There are a few guidelines that we suggest to minimize potential problems:

- The average length should be 8-10 amino acids
- Since the peptide is linked through C-terminus, the MAP technique favors N-terminal or internal peptides and is not recommended for peptides from the extreme C-terminus. The carrier attachment may also cause some steric hindrance.
- Cysteines can be problematic since disulfides can form non-specifically and C-terminal prolines give poor yields with this chemistry.
- MAP peptides behave much like large proteins. The high molecular weight of the complex does not lend itself to good quality control measure. Nevertheless, they possess high antigenicity and are good immunogens.

REFERENCES:

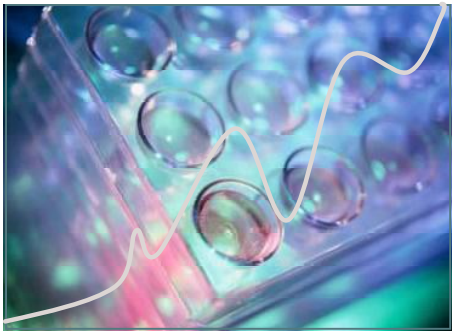
1. Posnett, D., McGrath, H., and Tam, J.P. A novel method for producing anti-peptide antibodies, *The Journal of Biological Chemistry*, 263, 1719-1725 (1988),.
2. Tam, J.P., Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system, Proc. *Natl. Acad, Sci.*, 85, 5409-5413 (1988).

For more product information visit: www.biosyn.com

ECONOMY CLASS

Features:

- MAP Synthesis up to 10 residues
- Immunization of 2 animals
- ELISA Testing
- ~100 ml crude serum
- 10-12 weeks delivery
- MAP peptide shipped to customer



ANTIBODY PRODUCTION

Custom Immunology Products

RABBIT ANTISERUM PACKAGE

- **Customer - supplied antigen**
  (1-2 mg of protein sample)
- **Immunization of 1 rabbit**
- **~50 ml crude serum**
- **ELISA Testing**
- **10-12 weeks delivery**
- *Post-Project maintenance is available for an additional fee*

SHEEP/GOAT ANTISERUM PACKAGE

- **Customer - supplied antigen**
  (1-2 mg of protein sample)
- **Immunization of 1 sheep or goat**
- **~250 ml crude serum**
- **ELISA Testing**
- **12-15 weeks delivery**
- *Post-Project maintenance is available for an additional fee*

ANTIBODY PURIFICATION

- IgG Purification (5ml serum or ascites fluid)
- IgG Purification (100 ml hybridoma supernatant)
- IgM Purification (5ml serum or ascites fluid)
- IgM Purification (100 ml hybridoma supernatant)

ELISA

This is provided after anti-peptide sear is harvested, together with pre-bleed serum. A 96 well peptide-coated micro-titer plate is used to determine the titer of the serum. Price is per rabbit

EPITOP SPECIFIC PURIFICATION

The high titer serum pool can be affinity purified on a peptide matrix. Bio-Synthesis will determine the appropriate peptide to be used for this purpose in consultation with the customer. We will purify the first 10 lots of your choice.

LABELING

- Horseradish Peroxidase (HRP)
- Alkaline Phosphatase
- B-Galactosidase
- Biotinylation

FLUOROCHROME CONJUGATION

- FITC Antibody Conjugation
- Rhodamine Antibody Conjugation
- Phycoerythrin (PE Antibody Conjugation)
- CY3 Antibody Conjugate

AFFINITY GEL

- Protein Antibody Affinity Gels
- Peptide Affinity Gels

PEPTIDE ANTIBODIES

Protocol...

ANIMALS:
Two New Zealand White Females, approximately 12 weeks of age, approximately 2 kg in weight

ADJUVANTS:

Primary Injection:
(1:1) 200 µg of conjugated peptide (app. 5ml of conjugate solution) Plus equal amounts of Freund's Complete

Booster Injection:
(1:1) 200 µg of conjugated peptide (app. 5ml of conjugate solution) Plus equal amount of Freund's Incomplete

STANDARD SCHEDULE

Day	Procedure	Amounts/Volumes per Rabbit
0 (week 0)	Pre-bleed Primary Injuection	2.0 - 5.0 ml of SERUM(1:1) conjugate solution: Freund's Complete
14 (week 2)	First-Booster	(1:1) conjugate solution: Freund's Incomplete
28 (week 4)	Second Booster	(1:1) conjugate solution: Freund's Incomplete
42 (week 6)	First Production bleed Third Booster Perform ELISA	20 ml of SERUM (1:1) conjugate solution: Freund's Incomplete
56 (week 8)	Second Production Bleed Fourth Booster	20 ml of SERUM (1:1) conjugate solution: Freund's Incomplete
70 (week 10)	Third Production Bleed	20 ml of SERUM

At the end of the 70 day protocol, the researcher is contacted to determine:

1. ***Continue project on a month-to-month basis***
2. ***Terminate project***
3. ***Project exsanguination***

Call **800-227-0627** or write to us at **biosyn@biosyn.com** for details

HINTS FOR SUCCESSFUL PEPTIDE ANTIBODY PRODUCTION

PEPTIDE SEQUENCE SELECTION GENERAL RULES

1. Examine N– and C-terminus of the protein. If they are hydrophilic, they often constitute an excellent choice.

2. If both N– and C-terminus are suitable, choose internal hydrophilic region of the protein. Hydrophilic sequences have the best chances to reside at the surface of the protein and therefore to be accessible to the antibodies.

3. Avoid possible glycosylation or phosphorylation sites

4. Avoid a series of hydrophobic amino acids such as alanine, tryptophan etc.

5. Short synthetic peptides are quite flexible and are unable to mimic alpha helical sequences. However, run algorithms for predicting secondary structures and avoid regions which are strongly predicted alpha helical prior to peptide selection.

6. A peptide range between 10-20 amino acids is recommended. Shorter sequences have fewer chances to include good peptides, longer peptides tend to make stable secondary structures which are not necessarily the same in the protein of interest. Longer peptides also increase the chances of cross-reactions with other proteins.

7. Run homology searches of the selected peptides against proteins of the organism obtained from in order to exclude unwanted cross-reactions to other proteins as much as possible. Less than 4-5 identical amino acids in a continuous stretch are required to exclude a cross-reaction.

8. Carrier protein coupling strategy needs to be taken into account. Alternatively, use the MAP strategy for synthesis of the peptide (not suitable for peptides from the C-terminus).

    - Peptides from the C-terminus of the protein should be linked to the carrier through the N-terminus.
    - Peptides from the N-terminus of the protein should be linked to the carrier by their C-terminus.
    - Internal peptides should be coupled at the less hydrophilic end.
    - N-terminal coupling is specific only if the peptide does not contain lysine residue.
    - C-terminal coupling is specific only if the peptide does not contain aspartyl or glutamyl residues.
    - If no specific coupling can be done, add cysteine at one end of the peptide. The only restriction is there should not be any other cysteines in the sequence.

9. Conjugation of a peptide to a carrier protein containing multiple epitopes, such as Keyhole-Limpet Hemocyanin (KLH) or Bovine Serum Albumin (BSA), are most common. KLH is preferred since it is more antigenic in the majority of animals. BSA is often used as a blocking reagent in assays, thus an anti-peptide antibody raised against a BSA conjugated peptide will show some specificity towards the buffer reagents. This can result in a false positive signal.

10. MAP is simply an alternative to KLH conjugation. It is recommended only for shorter peptides located either internally within a protein or at the N-terminus. KLH conjugation is recommended for a peptide at any location in a protein and when there are a cysteine residues within the peptide sequence.

11. Use at least 2 animals for each peptide since there is some genetic variability between animals.

12. Use more than 1 peptide for each protein in order to increase the chances for antibodies to recognize the protein.

The services for the separation of the antibodies produced against the individual peptides are available at **Bio-Synthesis**, call **800-227-0627** or write to us at **support@biosyn.com** for FREE consultation on peptide selection.

SYNTHESIS WITH A DIFFERENT APPROACH

BIOPNA SYNTHESIS

BIOCONJUGATIONS

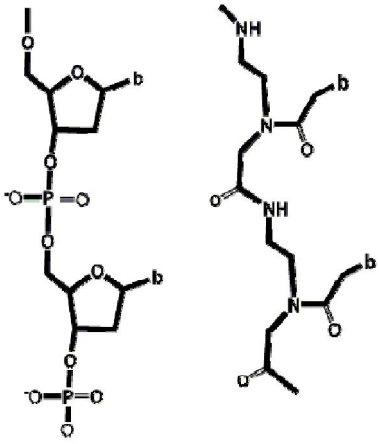
CREATIVITY AND INNOVATION

A CUSTOMIZED SOLUTION FOR PEPTIDE NUCLEIC ACID SYNTHESIS

With the growing need to improve nucleic acid binding interactions, peptide-like nucleic acid is finding increased use in a number of biological applications, i.e. control of gene expression, better specificity in DNA diagnostic applications, etc., all of which are unavailable with DNA probes. Instead of a sugar phosphate backbone of natural nucleic acid, this unique peptide-like DNA structure contains a synthetic peptide backbone. This is formed by N-(2-amino ethyl)-glycine units and different bases (purines and pyrimidines), which are linked to the backbone by methylene carbonyl linkages; results in an achiral and uncharged mimic. Unlike DNA or DNA analogs, this peptide-like DNA structure does not contain any pentose sugar moieties or phosphate groups. This structure is chemically stable and resistant to hydrolytic reactions and, thus, not expected to be degraded inside a living cell.

ADVANTAGES OF PEPTIDE NUCLEIC ACID

- Peptide nucleic acid is capable of sequence specific recognition of DNA and RNA. The hybrid complexes exhibit extraordinary thermal stability and unique ionic strength.
- Greater stability due to its "unnatural" structure, this pseudo-peptide nucleic acid is resistant to degradation and modification by nucleases, proteases, pH and temperature; indefinite shelf life is possible.
- The neutral backbone of peptide nucleic acid creates stronger binding independent of salt concentration, allowing more robust hybridization applications.



visit www.biosyn.com

BIOPNA SERVICE FEATURES

- Scale of 2 umole - 0.1 molar
- Analysis by HPLC, >85% pure
- Characterized by MALDI-TOF Mass Spec
- Certificate of Analysis

Modification:
Biotin, FITC, addition of amino acids, addition of J bases, Bis PNA(PNA-Linker-PNA), Tris PNA (PNA-Linker-PNA-Liner-PNA)

Delivery:
Delivery time is dependent upon the complexity of the sequence. Estimated time of delivery is about 2-3 weeks, upon acceptance of an order, unless otherwise specified.

Bio-Synthesis, Inc. Toll Free: 800-227-0627 Tel: 972-420-8505 Fax: 972-420-0442 Web: www.biosyn.com Email: biosyn@biosyn.com

BIOCONJUGATES

Applications of synthetic oligonucleotide analogs and their peptide conjugates have, in recent years, been identified as valuable tools in molecular biology. In particular, they have become increasingly important due to the identification of peptides as viable carriers for enhancing the cell delivery of oligonucleotides. BSI offers several **BIOCONJUGATES** such as:

- **PNA - Peptide Conjugates**
- **PNA - DNA Chimer**
- **Peptide - DNA**
  - -Bi-functional Linkages (MBS or SWCC)
  - -Disulfide Linkage
  - -Thioether Linkage
  - -and more..

BIOPNA / BIOCONJUGATE TECHNICAL NOTES

SEQUENCE DESIGN FOR BIOPNA

Length: 12-17 bases optimal length excluding linker, amino acids and labels
Amino Acid Content: Any amino acid up to 30 residues
Purine Content: Limit to 60%, avoid 4G's or 6 purines in a row
Avoid Self Complementary: Palindromes, hairpins, inverse repeats

SOLUBILIZING AND STORAGE

These pseudo peptide DNA oligomers are readily soluble in aqueous solutions at pH 6.0 or lower, and less soluble at higher pH values. Typical concentration in various applications is 0.05 - 10µM. For most applications, a final assay concentration ≤1 µM is suitable.

Some oligomers are more difficult to solubilize because of self-aggregation and subsequent precipitate. If the solution is cloudy or contains aggregates after solubilization, we recommend additional heating at 50°C for 10 minutes. Briefly centrifuge to bring down any condensate or add N-Methylpyrrolidone (NMP) to a final concentration of 10-50% (v/v). First vortex, then pipette any visible aggregates up and down. For high purine sequences (>50%), or oligomer concentrations >500µM, use 50% NMP. We recommend always heating to 65°C immediately prior to use, to ensure that aggregation is minimized.

DO NOT store this peptide-DNA oligomer in neutral buffer, store at –20°C in a lyophilized form in a sealed storage box containing desiccant

DESIGN FOR BIOCONJUGATES

Both peptide and oligonucleotide units are assembled separately, on their own supports, using conventional synthesizers and methodologies. The conjugate of the two molecules can be accomplished through solution phase coupling strategies by one of the linkages such as the disulfide, maleimide, thioesther, or amide bond. Ready to use amino linkers and thiol linkers for oligonuceoltide 3' and/or 5' end modification are required. Peptides can be functionalized by adding a cysteine residue at the N-terminus, C-terminus, or even at an internal position. Peptides and oligonucleotides are then joined together by bi-functional cross linkers.

For more information, please contact us at **support@biosyn.com**

Intellectual and commercial rights for clinical/therapeutical diagnostics of PNA's covered by patents owned by Isis Pharmaceuticals, San Diego, CA and ABI, Palo Alto, CA.

THE POSSIBILITIES ARE INFINITE

CONTRACT ORGANIC *SYNTHESIS*

INTEGRATED, INNOVATIVE SOLUTIONS... THE POSSIBILITIES ARE INFINITE

With over two decades of combined experience, our team of Ph.D organic chemists have expertise in a wide variety of chemistries from intermediates, reference compounds, and starting materials, to derivatives of lead compounds. We offer process development from laboratory to production scale. For collaborative custom projects, we offer synthesis services of compounds that are not commercially available (at the mg, gram or kilogram scales). Our project management team will conduct a detailed investigation of your needs and synthesis specifications to insure success.

COLLABORATIVE CUSTOM PROJECTS

The customer should provide literature, methods, or their own synthesis route describing all details. The more information provided, the shorter the project timelines and the lower the cost. Our staff of chemists will evaluate synthesis feasibility prior to the initiation of the project, and provide an estimate of cost and delivery time.

CONTRACT RESEARCH

Contract research is undertaken when synthetic information is limited, the project scope is major, for scale up efforts, patent portfolio development, or other purposes. In these instances, **Bio-Synthesis** will determine the best synthetic approach along with the requestor's priorities/ needs. Prior to project execution, our staff chemists will conduct pilot runs to determine feasibility and an initial set-up fee will be charged. Once a pilot project is completed, a research agreement is drawn up. All projects are conducted under strict confidentiality.

Write to us: biosyn@biosyn.com

PROJECT PORTFOLIOS

*For the past two decades, **BSI** has synthesized various types of synthetic compounds. Our project portfolio includes, but is not limited to...*

- **Multi-Step Synthesis**
- **Peptide Building Blocks**
- **Inositol Phosphates**
- **Imidazole Derivatives**
- **Thiadiazolidinone Derivatives**
- **Pharmaceutical Intermediates**
- **Custom Bioconjugates**
- **Lipid and Phospholipids**
- **Patent Development**
- **And Other Biologically Active Structures**

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FEATURES AND CAPABILITIES

RESEARCH AND DEVELOPMENT

With over twenty years of success in synthesis, **Bio-Synthesis** provides one-stop integrated solutions with a wide range of problem-solving expertise in chemical synthesis, supported by world-class analytical capabilities. The result is fast, reliable and efficient project execution. Whether it is developing a new process or improving an existing one, **BSI** can provide solutions to meet your needs.

CUSTOM SYNTHESIS FROM MILLIGRAMS TO MULTI-GRAMS

When there is a need for various quantities of material, contact **BSI**. Our staff has a proven track record of preparing a wide range of compounds on an equally wide range of scales. As your requirements expand, our process management team will construct a detailed production plan to meet your needs in a timely and cost-effective manner. Since the initial pilot plan used to prepare a few milligrams may not be suitable to prepare tens to thousands of grams, our staff will adapt and develop a production process to fulfill your expectations.

SCALE-UP FROM AN EXISTING PROCESS

Our laboratory is set up to reproduce large-scale syntheses in a timely and efficient manner, while using our customer's demonstrated synthesis route(s). We will identify and develop an appropriate scale-up solution to meet your needs.

PROCESS RESEARCH AND DEVELOPMENT

Over the years, we have assisted pharmaceutical companies in developing new and proprietary routes to existing compounds or scalable processes using our talented chemists and state-of-the art technology. This includes statistical design of experiments, real-time monitoring to generate detailed reaction/impurity profiles, and further process development.

For all Contract Organic Synthesis,
*Call **800-227-0627** or write to us at **biosyn@biosyn.com***

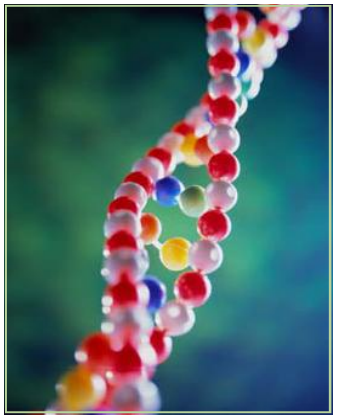
HIGH THROUGHPUT SYNTHESIS

CUSTOM DNA SYNTHESIS

EXPERIENCE OUR QUALITY, SPEED AND INNOVATION
HIGH THROUGHPUT CUSTOM DNA SYNTHESIS

Since 1984, **Bio-Synthesis**, the first company to provide commercial custom oligonucleotide synthesis services, has remained a major force in advancing biotechnology research both as leading supplier and developer of new technologies for oligo synthesis. Researchers choose **BSI** as their major supplier for oligonucleotide synthesis because of our core values: **QUALITY**, **SPEED** and **HIGH THROUGHPUT** capabilities. Quality is not just a goal, it is a **GUARANTEE**. Every oligo synthesized must pass strict quality control guidelines before delivery to our customers. In addition to high quality production, speed of our oligo synthesis has increased to next day delivery in most cases. Through improvement from traditional chemistry to development of advanced state-of-the-art proprietary instrumentations, **BSI** has achieved the goal of providing the most economical, high throughput, quality custom DNA synthesis.

For more information, Call 800-227-0627 or visit www.biosyn.com



DNA SYNTHESIS

- **Standard DNA Synthesis**
- **Purification**
- **Long Oligo Synthesis**
- **High Throughput 96-Well-Formatted Oligos**
- **Phosphorothioate Oligos**
- **Chemically Modified Oligos**
- **DNA Markers**

Quality Assurance

Every oligo synthesis is verified under strict quality control either by MALDI-TOF mass spectrometry or polyacrylamide gel eletrophoresis (PAGE) analysis. Final yield is determined by using UV absorbance at OD260. A quality assurance certificate, which includes: ug/OD, MELTING TEMPERATURE, and MW is provided with every oligo. *We guarantee all oligo sequences as customers have specified*. Otherwise, we will replace the oligos immediately at no charge, within 30 days of shipment.

STANDARD DNA SYNTHESIS

Scale	Quantity	Order Specification	Turnaround
*10 nmole	5 ODU	**Up to 45 bases**	1-2 days
50 nmole	10 ODU	**Up to 45 bases**	1-2 days
200 nmole	20 ODU	**Up to 100 bases**	1-2 days
1.0 µmole	40 ODU	Up to 45 bases	2-3 days
5.0 µmole	200 ODU	Up to 45 bases	2-3 days
10.0 µmole	400 ODU	Up to 45 bases	2-3 days

MODIFICATIONS

PHOSPHORYLATION
- 5′ - phosphorylation
- 3′ - phosphorylation

BIOTIN
- Biotin
- Biotin dT
- 5′-Biotin
- 3′-Biotin

DABCYL & DNP Labeling
- 3′-Dabcyl
- 2,4-Dinitrophenyl (DNP)

FLUORESCENT LABELS
- Texas Red
- TAMRA
- CY3, CY5
- Psoralen C2 or C6

DIGOXIGENIN
- Digoxigenin 5′

ABI DYES
- 5′ or 3′-FITC
- 6-FAM
- HEX
- TET
- And many others...

3′-Modifications
- 3′Amino-Modifier C3
- 3′-Amino-Modifier C7 Glyceryl
- 3′-Thiol-Modifier C3 S-S
- 3′-Carboxylate Photolabile
- 3′-Amino Photolabile C6

5′-Terminus Modifications
- 5′-Amino-Modifier C3-TFA
- 5′-Amino-Modifier c6
- 5′-Amino-Modifier C6-TFA
- 5′-Amino-Modifier C12
- 5′-Amino-Modifier 5
- 5′-Thiol-Modifier C6, C6 S-S

Other Modifier...
- Chain Terminator
- Structure Modifications
- Halo-genated Modification
- Mutagenesis Modifications
- Convertible Bases
- Therapeutic Bases
- Cyclic Oligonucleotides
- Sequence Modifications
- Spacer Modification
- Other Bases
- Duplex Effects

*Call for items not listed above at **800-227-0627** or write to us at **biosyn@biosyn.com** for a price quotation.*

**Post-synthesis modifications may yield 50% less than the below stated values.*

PURIFICATIONS FOR STANDARD DNA SYNTHESIS

Purification	Scale	Quantity
Cartridge	10 nmole	NA
	50 nmole	NA
	200 nmole	6 ODU
	1.0 µmole	15-20 ODU
	5.0 µmole	>100 ODU
	10.0 µmole	Inquire

Purification	Scale	Quantity
HPLC	10 nmole	NA
	50 nmole	NA
	200 nmole	4-5 ODU
	1.0 µmole	10-15 ODU
	5.0 µmole	>100 ODU
	10.0 µmole	Inquire

Purification	Scale	Quantity
PAGE	10 nmole	NA
	50 nmole	NA
	200 nmole	3-4 ODU
	1.0 µmole	5-10 ODU
	5.0 µmole	>30 ODU
	10.0 µmole	Inquire

**Guarantee is for 20 mers or longer. Shorter oligos may have fewer ODs.*

USEFUL DNA NOTES

RECOMMENDED APPLICATIONS FOR PURIFICATION

CARTRIDGE	PCR, probing antisense, mobility shift, arbitrary 10-mer applications, hybridization plus gene synthesis, primer extension, sequencing, DNA fingerprinting, cycle sequencing, RT-PCR, in situ hybridization, microsatellite polymorphisms. Recommended for oligo <30 mers
HPLC	PCR, probing antisense, mobility shift, arbitrary 10-mer applications, hybridization. Recommended for oligo <50 mers
PAGE	PCR, probing antisense, mobility shift, arbitrary 10-mer applications plus gene synthesis and primer extension. Recommended for oligo >35 mers

DEFINITION OF 1 O.D. AT 260

1.0 O.D. A260 refers to the amount of oligonucleotide which, when dissolved in 1 ml of water, typically results in an absorbance of 1 when measured at 260 nm in a 1 cm path length cuvette. The actual concentration can range from 39mg/ml for a homopolymer of C to 20ug/ml for a homopolymer of A. For most practical experiments, 1 O.D. represents approximately 33 ug of oligo with an equal mixture of the four bases.

OLIGO CONVERSION FACTORS

1 O.D. A260 unit of an oligo = 33 ug/ml

RESUSPENSION BUFFERS

1. Sterile Water (ddH2O)
2. TE buffer (10 mM Tris-HCL,1mMEDTA)pH7.5

DNA STORAGE CONDITIONS & STABILITY

Lyophilized (-20°C) = 6 months to several years
Lyophilized (25°C) = 2 months to 1 year
Dissolved (-20°C) = 1 to 6 months
Dissolved (25°C) = 1 week to 3 months

MOLECULAR WEIGHT OF AN OLIGO

((A X 312.2)+(GX328.2)+(CX288.2)+(TX303.2)-61) where A,C, G, T represent the # of A's, C's, G's and T's in an oligo.

MELTING TEMPERATURE FOR NUCLEIC ACID HYBRIDIZATION

Up to 25 bp:

Tm=4°C (G+C) + 2°C (A+T)

More Than 25 bp:

Tm= 81.5°C + 16.6 logM + 0.41(%G+C)-500/n-0.61 (% formamide)

(M=[NA+] in moles/ liter; n=length of shortest chain in duplex)

OD UNITS TO NANOMOLES

 # OF NMOLE OF OLIGOS = 100 X O.D A260/LENGTH

Length	1 OD	5 OD	Length	1 OD	5 OD
10	10.nm	50.0 nm	**21**	4.8 nm	23.8 nm
11	9.0 nm	45.0 nm	**22**	4.5 nm	22.7 nm
12	8.3 nm	41.7 nm	**23**	4.3 nm	21.7 nm
13	7.7 nm	38.5 nm	**24**	4.2 nm	20.8 nm
14	7.1 nm	35.7 nm	**25**	4.0 nm	20.0 nm
15	6.7 nm	33.3 nm	**26**	3.8 nm	19.3 nm
16	6.3 nm	31.3 nm	**27**	3.7 nm	18.5 nm
17	5.9 nm	29.4 nm	**28**	3.6 nm	17.9 nm
18	5.6 nm	27.8 nm	**29**	3.4 nm	17.2 nm
19	5.3 nm	26.3 nm	**30**	3.3 nm	16.7 nm
20	5.0 nm	25.0 nm			

PAGE PURIFICATION INFORMATION

(DYE MIGRATION DENATURING GEL UREA), 1X TBE

%gel	Separation range		Migration rates
Blue	Xylene Cyanol		Bromophenol
5.0	10-200	35	140
8.0	80-150	20	75
12.0	40-100	12	53
15.0	12-80	10	44
20.0	8-60	8	24

DELIVERY

Standard oligos are normally shipped 24-48 hours after receipt of a purchase order. Most modified or purified oligos are shipped within 3-5 days. Our customer service is available to answer any questions regarding order status, M-F 8:00 - 6:00 pm CST.

For more technical questions, contact us at
support@biosyn.com

QUALITY CONTROL ON-DEMAND

ANALYTICAL SERVICES

Since routine analytical analyses are mandated for all of **Bio-Synthesis**'s custom products, we provide proteomic analytical services to our customers as an independent means for validating their compounds or materials. Our analytical services include:

- **PEPTIDE PROTEIN SEQUENCING**
- **AMINO ACID ANALYSIS**
- **MASS SPECTRAL ANALYSIS**

PEPTIDE PROTEIN SEQUENCING

STATE-OF-THE-ART-TECHNOLOGY High sensitivity; capable of sequencing at low picomole levels.
FLEXIBILITY AND RELIABILITY Sequence protein in solution, gel or PVDF blots.
EXPERTISE AND EXPERIENCE A proteomic Ph.D. expert oversees every analysis.
SIMPLE AND EASY Sample submission steps
FAST AND ECONOMICAL Low cost with results in 2 weeks

N-TERMINUS SEQUENCING
INTERNAL SEQUENCING
SAMPLE PURIFICATION

AMINO ACID ANALYSIS

Amino acid analysis is a suitable tool for precise determination of protein quantities, but also provides detailed information regarding relative amino acid composition and free amino acids. The relative composition of the amino acid gives a characteristic profile of a protein, which is often sufficient for identification. The procedure involves:

- **Hydrolysis**
- **Separation, Detection and Quantification**

MALDI (matrix-assisted laser desorption/ionization)

A laser-based soft ionization method has proven to be one of the most successful ionization methods for mass spectrometric analysis and investigation of large molecules. For demanding research, **BSI** offers top-quality services with:

- High sensitivity
- High resolution
- Fast, accurate and reliable results

USEFUL NOTES FOR SAMPLE PREPARATION

RECOMMENDED NUMBER OF DEGRADATION STEPS

- 5 residues - confirming N-terminus or recombinant protein
- (target sequence needs to be provided)
- 10-15 residues - Protein ID of an unknown protein
- 10-20 residues - Design of an oligonucleotide probe
- >10 residues - De novo sequencing of an unknown protein

Chemicals to Avoid

1. **Samples should be free of primary amines (i.e., Tris or Glycine):** Tris and glycine are common in samples recovered from SDS-PAGE and can be removed by ethanol-precipitation, trichloroacetic acid precipitation, reverse-phase chromatography, gel filtration, ion-exchange separation, Centri-Plus concentrator.
2. **Glycerol and sucrose:** These compound are commonly added to buffers for storage and handling. They are not volatile and leave highly viscous residues.
3. **Nonionic detergents:** Triton X-100, Brij, and Tween solutions often contain aldehydes, oxidants and other contaminates that can inhibit Edman degradation
4. **SDS:** Large quantities of SDS can cause instrument malfunction and may lead to the loss of sample from the filter.

NOTE: *Dialysis tubing is often a source of contaminants and other interfering substances. Avoid dialysis as a last step in sample preparation or use thoroughly cleaned, high-quality tubing. Always dialyze against a salt counterion or dilute acid to prevent the protein and contaminates that may be present from sticking to the tubing.*

Sample Preparation

1. All reagents and solvents must be of the highest purity available to avoid contaminating the substance. HPLC grade, sequencing grade and electrophoresis grade reagents are recommended; AVOID using molecular biology grade reagents.
2. Always wear gloves. Dust and finger prints are a major source of amino acids contamination present in sequencing samples.
3. Avoid lyophilized sample in glass tubes, this can lead to loss of sample for some proteins. Sample volumes should be less than 150 µl.
4. Samples should be in a volatile solvent or buffer such as acetic acid, TFA, water, propanol, acetonitrile, pyridine or ammonium bicarbonate (if lyophilized repeatedly). Minimum of 10-100 pmol is preferred, although a lower amount is acceptable, it is more practical to sequence larger amount of protein to be confident of the sequence obtained, or if n-terminus is blocked then internal sequencing may be recommended. In most cases the amount of sequenceable material is underestimated by sample loss, inaccurate quantization, or N-terminal blockage during sample preparation.
5. Avoid large amounts of detergent; must be less than 0.1% SDS.

Cysteine Modification: Cys should be modified before sample submission since unmodified Cys residues cannot be detected.

N-terminal blockage: If the amino terminus is blocked, the protein or peptide cannot be sequenced using Edman degradation. We perform de-blocking procedures and will discuss options with our customers if N-terminal blockage is suspected.

Glycosylation and other Modifications: Glycosylated amino acids and phosphorylated amino acids may results in blank cycles, reduced peaks or altered retention times.

Protein Sample in PVDF:
PVDF membranes with a pore size of 0.2 micron are preferred for sequencing. The protein can be stained with Coomassie Blue R-250, Amido Black, Ponceau S or Sypro Ruby. DO NOT use silver stain. Coomassie blue is sensitive enough to detect 50-200 ng of protein on PVDF (Note: nitrocellulose membranes are not compatible with the reagents and organic solvents used in automated protein sequencing). Following transfer, rinse the membrane 3-5 times with HPLC water to reduce the left over Tris and glycine contaminates. The size of an excise piece of PVDF membrane containing the protein band should be smaller than 40 mm2. As a rule of thumb, if the Coomassie blue stained protein band on the blot is visible on a photocopy, then there is enough material for sequencing.

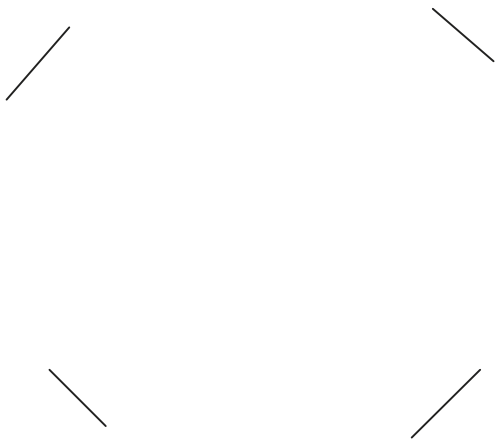
Protein Sample in solutions:
A protein sample may also be submitted dissolved in 150 µl or less of a suitable solvent, such as HPLC water, 0.1% to 50% TFA, 5% acetic acid or aqueous acetonitrile.

NOTE: More than half of all eukaryotic intracellular proteins have blocked amino-termini and cannot be sequenced directly. Artificial blocking of the amino-terminus may also occur during purification. The blocking group must be removed (if possible) before sequencing.

GENERAL INFORMATION FOR AMINO ACID ANALYSIS

1. All Samples for amino acid analysis should be salt-free. Contamination of samples with non-volatile primary amines (Tris, glycine), salts, or detergents affect the accuracy analysis. These materials may be removed by ethanol-precipitation, trichloroacetic acid precipitation, reverse-phase extraction, gel filtration, ion-exchange separation, or dialysis.
2. Some amino acids, such as Ser, Thr and Tyr, are partially degraded under hydrolysis conditions. These amino acids are estimated by extrapolating experimental values to time zero.
3. Amino acids, such as Ile, Leu and Val, are released slowly during hydrolysis because peptide bonds in these residues are more resistant to hydrolysis. The amounts of these amino acids are estimated by the values obtained after 75-hrs of hydrolysis.
4. Cystine and cysteine decompose under normal hydrolysis conditions. These amino acids may be converted to stable derivatives, such as carboxymethyl cysteine, pyridylethyl cysteine, and cysteic acid before hydrolysis. Alternatively, 1/2 Cys is converted to a mixed-disulfide derivative during the hydrolysis reaction.
5. Tryptophan decomposes under normal hydrolysis conditions. Therefore, tryptophan analysis can be carried out after hydrolysis in 3M mercaptoethanesulfonic acid. Because tryptophan residues are scarce in protein, tryptophan analysis requires a larger amount of sample material than for other amino acids. Tryptophan analysis is carried out only when requested.

For more technical questions, contact us at
support@biosyn.com



Bio-Synthesis, Inc. Toll Free: 800-227-0627 Tel: 972-420-8505 Fax: 972-420-0442 Web: www.biosyn.com Email: biosyn@biosyn.com

Customized solutions...
designed just for you



BIOSYNTHESIS INC
SYNTHESIS THROUGH INGENUITY

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Your source for custom DNA, Peptides, Antibodies, Contract Organic Synthesis , Analytical Services and Molecular Biology Products