Testing and Evaluation of Synthesized Peptides using Quality Metrics

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INTRODUCTION

Chemical synthesis and in vitro folding of peptides can lead to products that contain large amounts of inactive material, the proportion of which varies from lot to lot. These variations can impact research as the consistency of results commonly correlates with the quality of the material under study. Further complicating matters for researchers, the suppliers for these peptides of interest tend to be small operations with unknown quality control processes, and many are located OCONUS. To mitigate these inconsistencies, DEVCOM Chemical Biological Center (CBC) has developed a program to document, produce, and characterize synthetic lots of peptides using standards and practices for testing under CBC's established Quality program. Focusing on Quality metrics, CBC implemented inventories that include equipment identification, vendor and suppliers, and material receipt and tracking which culminates in an all-inclusive quality document master list. These lists capture and record the metrics of all equipment and material required for the standardization and reproducibility of synthesized peptide lots. Furthermore, operating procedures for the equipment as well as the establishment of test methods bolster and drive a standardized system while still allowing documented deviations that occur in research settings. All material produced is subjected to testing under approved specifications that examines sample purity and activity. Product shelf-life is also evaluated to ensure that material does not have a significant loss of activity over time and is a predicter to expiration dating. Lastly, a shipping study outlines how the material is packaged and shipped during diverse temperature conditions (30◦F and 90◦F) to assess the effects of temperature on the purity and activity of a peptide sample. This allows for a better understanding of the stability of the material during transit. Production and testing under quality type documentation and material tracking enables the standardization across facilities ensuring consistencies in quality and performance of the material between producers. Establishing quality metrics and documentation facilitates transition where all producers can synthesize and test material resulting in the same analytical result and performance.

QUALITY CONTROL

ISO/IEC 17025 ACCREDITATION

DEVCOM CBC Biomanufacturing Branch maintains International Standards for ISO/IEC 17025 accreditation for several methods listed on the Scope of Accreditation.

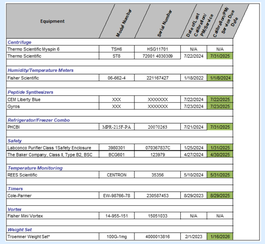
DEVCOM CBC @ DTRA CBD S&T Conference

Scan the QR Code to view all of CBC's 2024 DTRA CBD S&T Conference materials
https://cbc.devcom.army.mil/cbdst-conference/

TYPES OF DOCUMENTATION

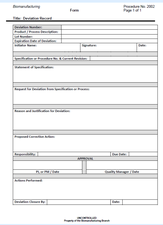
Traditional laboratory notebooks are great to use when work could lead to a patent. Although notebooks follow a general guideline as to what information should be captured in an experiment, it is ultimately up to the user in what information is captured. This can prove difficult when other scientists try to reproduce the same experiment. Establishing standardized documents can ensure the same method is used every time. Standardizing documentation does not mean that the user can't deviate from an approved method. Users can deviate from an approved method by recording that information on a deviation form. The document can be revised later ensuring the most up to date method is being used. Types of documentation that supports standardization and reproducibility are the following: Internal Operating Procedures, Forms, Vendor Supplier Lists, Equipment Lists, and Material Receipt and Inspection Forms to name a few.

EQUIPMENT LIST



Equipment	Model Number	Serial Number	Date Last Serviced / Calibrated	Date Next Service / Calibration Due
Centrifuge				
Thermo Scientific Mysapin 6	TS40	WS011791	N/A	N/A
Thermo Scientific	075	72001-402039	7/23/2024	7/31/2025
Humidity/Temperature Meters				
Fisher Scientific	06-662-4	221187427	1/18/2022	1/18/2024
Peptide Synthesizers				
CEM Liberty Blue	XXX	XXXXXXXX	7/22/2024	7/22/2025
Gyros	XXX	XXXXXXXX	7/23/2024	7/23/2025
Refrigerator/Freezer Combo				
PHCBI	MPR-215F-PA	29076243	7/21/2024	7/31/2025
Safety				
Labconco Purifier Class 1Safety Enclosure	3960301	67539760701	1/25/2024	1/31/2025
The Baker Company, Class II, Type B2, 6SC	BCSB01	12979	4/27/2024	04/30/2025
Temperature Monitoring				
REES Scientific	CENTRON	35356	5/15/2024	5/31/2025
Timers				
Cole-Parmer	EW-66766-70	23958743	8/29/2023	8/29/2025
Vortex				
Fisher Mini Vortex	14-955-151	15851033	N/A	N/A
Weight Set				
Troemner Weight Set*	1000-1mg	4000013816	2/1/2023	2/15/2025

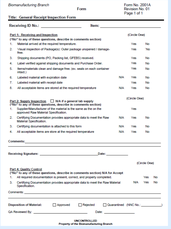
MATERIAL RECEIPT AND INSPECTION FORM



VENDOR AND SUPPLIER LIST

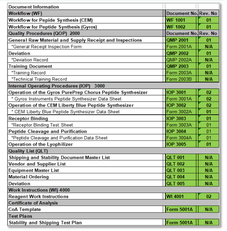
Company	Address	POC	Telephone	Web Address (if applicable)
CEM Corporation	3100 Smith Farm Road Matthews, NC 28104	Customer Service	(800) 726-3331	https://cem.com/
Cole-Parmer	625 Bunker Court Vernon Hills, IL 60061-1844	Customer Service	(800) 323-4340	http://www.coleparmer.com/
DNJ Services, Inc	2950 Bergey Road, Suite A Hatfield, PA 19440	Customer Service	(800) 882-9108	www.e-mechanics.com
Fisher Scientific	2000 Park Lane Pittsburgh, PA 15275	Customer Service	(800) 766-7000	www1.fishersci.com https://www.coleparmer.com/shop
Gyros Protein Technologies	Tucson, AZ 85714	Customer Service	0 (888) 31662	
Integrated Sample Solutions	1603 Bustard Road, Lansdale, PA 19446	Customer Service	(610) 287-3000	www.integratedsamplesolutions.com
Fisher's Instrument Co.	Penn Road, Box 4026 Hatboro, MA 19040-4026	Customer Service	800-472-6446	www.rainin.com
REES Scientific	1007 Whitehead Road Extension Trenton, NJ 08638	Customer Service	(800) 257-3141	www.reesscientific.com
Sigma Aldrich	PO Box 14508 St. Louis, MO 63178	Customer Service	800-325-3010	http://www.sigmaaldrich.com/united-states.html
Singer Scientific	2301 Service Rd. Beloel, QC, J2G 4S5, Canada	Customer Service	450-641-1721	ss@singer.com
Thermo Scientific	5747 N. Meridian Rd. Rockford, IL 61101	Customer Service	(800) 874-3723	http://www.thermoscientific.com (purchase is online)
Ultra	12075 Lime Drive Rancho Penas, MA 92315	Customer Service	1-909-390-5515	https://www.ultra.com/
VWR	100 Matsonford Road, Radnor, PA 19087	Customer Service	800-932-5000	www.vwr.com

DEVIATION FORM



MASTER DOCUMENT LIST

The master document list is a living document. Revisions to this list occur when documents are annually reviewed, changes to a process are made, or when additional documents are added or become obsolete. The list tells the user what the current version of the document is so that they have and are using the latest approved document while performing their task.



Document Information		
Workflow (WF)	**Document No./Rev. No**	
Workflow for Peptide Synthesis (CTM)	WF 1001	01
Workflow for Peptide Synthesis (Gyros)	WF 1002	01
Quality Procedures (QCP) 2000	**Document No./Rev. No**	
General Raw Material and Supply Receipt and Inspections	QRP 2001	02
"General Receipt Inspection Form	Form 2001A	N/A
Deviation	QRP 2002	01
"Deviation Record	QRP 2002A	N/A
Training Document	QRP 2003	01
"Training Record	Form 2003A	N/A
"Technical Training Record	Form 2003B	N/A
Internal Operating Procedures (IOP) - 3000		
Operation of the Gyros PurePrep Chorus Peptide Synthesizer	IOP 3001	02
"Gyros Instrument's Peptide Synthesizer Data Sheet	Form 3001A	02
Operation of the CEM Liberty Blue Peptide Synthesizer	IOP 3002	01
"CEM Liberty Blue Peptide Synthesizer Data Sheet	Form 3002A	01
Reception Binding	IOP 3003	01
"Reception Binding Test Sheet	Form 3003A	01
Peptide Cleavage and Purification	IOP 3004	01
"Peptide Cleavage and Purification Data Sheet	Form 3004A	01
Operation of the Lyophilizer	IOP 3005	01
Quality List (QL 7)		
Shipping and Stability Document Master List	QL 7 001	N/A
Vendor and Supplier List	QL 7 002	N/A
Equipment Master List	QL 7 003	N/A
Memo-in Ordering	QL 7 004	N/A
Deviation	QL 7 005	N/A
Work Instructions (WI) 4000		
Reagent Work Instructions	WI 4001	02
Certificate of Analysis		
CofA Template	Form 5001A	N/A
Test Plans		
Stability and Shipping Test Plan	Form 5001A	N/A

OPERATING PROCEDURES

Internal Operating Procedures (IOP) are created to standardize how equipment is run. Standardized documents ensure everyone is operating the equipment in the same manner. This includes forms that accompany the IOP to record reagents and critical steps within the process. Utilizing IOP's and forms ensures everyone is following the approved method and capturing the same information.

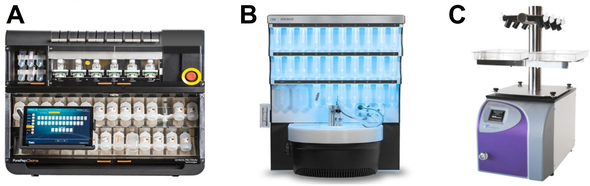


Figure #1. A. Gyros Peptide Synthesizer, **B.** CEM Liberty Blue Peptide Synthesizer, **C.** VirTis Benchtop Pro Freeze Dryer

CERTIFICATE OF ANALYSIS

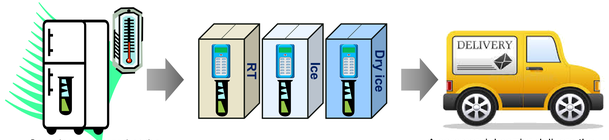
A Certificate of Analysis (CofA) is generated for each test sample that has successfully passed established specifications. Data generated over the course of testing is reported on the CofA along with the storage conditions. A CofA is accompanied with the material when the material is stored or shipped.

SHIPPING AND STABILITY

Approved test plans ensure that all parties are aware of and agree to the testing that will be performed, the methods that be will used, and the defined outputs desired at the completion of the study.

SHIPPING STUDY

A shipping study provides insight on how material is packaged and shipped during diverse temperature conditions (30◦F and 90◦F) to assess the effects of temperature on the purity and activity of a sample. Often packages are left sitting on a loading dock in the middle of summer or in the dead of winter before they reach their destination. Commercial carriers do not have the storage capacity to properly store these temperature dependent packages. A shipping study allows for a better understanding of the stability of the material during transit. Data loggers are placed within the package to record the temperature of the box that the samples are shipped in. Upon return, samples are tested and data compared to the original results at the time of initial production.



Samples are stored at the appropriate storage temperature. The temperature units are monitored 24/7 for power and temperature fluctuations.

Packages are prepared with samples at RT, Ice, and Dry Ice including the appropriate forms (SDS and CofA) with a data logger in each package.

A commercial carrier delivers the package. The receiver refreshes the packages containing ice and dry ice before it is shipped back to the sender for testing.

STABILITY TESTING

Product shelf-life is evaluated to ensure that material does not have a significant loss of activity over time and is a predicter to expiration dating. Pre-defined test points are defined in the test plan. Each test point utilizes a control that is treated exactly like the test sample.

	Stability Study		Shipping Study					
			Summer			Winter		
	Lyophilized	Resuspended	RT	Ice	Dry Ice	RT	Ice	Dry Ice
Month 0	Receptor Binding	Receptor Binding						
	Physiological Activity	Physiological Activity						
		MALDI Mass Spec.						
		LC/MS						
Month 3	Receptor Binding	Receptor Binding	Receptor Binding	Receptor Binding	Receptor Binding			
	Physiological Activity	Physiological Activity	Physiological Activity	Physiological Activity	Physiological Activity			
Month 6	Receptor Binding	Receptor Binding						
	Physiological Activity	Physiological Activity						
Month 9	Receptor Binding	Receptor Binding				Receptor Binding	Receptor Binding	Receptor Binding
	Physiological Activity	Physiological Activity				Physiological Activity	Physiological Activity	Physiological Activity
Month 12	Receptor Binding	Receptor Binding						
	Physiological Activity	Physiological Activity						
		MALDI Mass Spec.						
		LC/MS						

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