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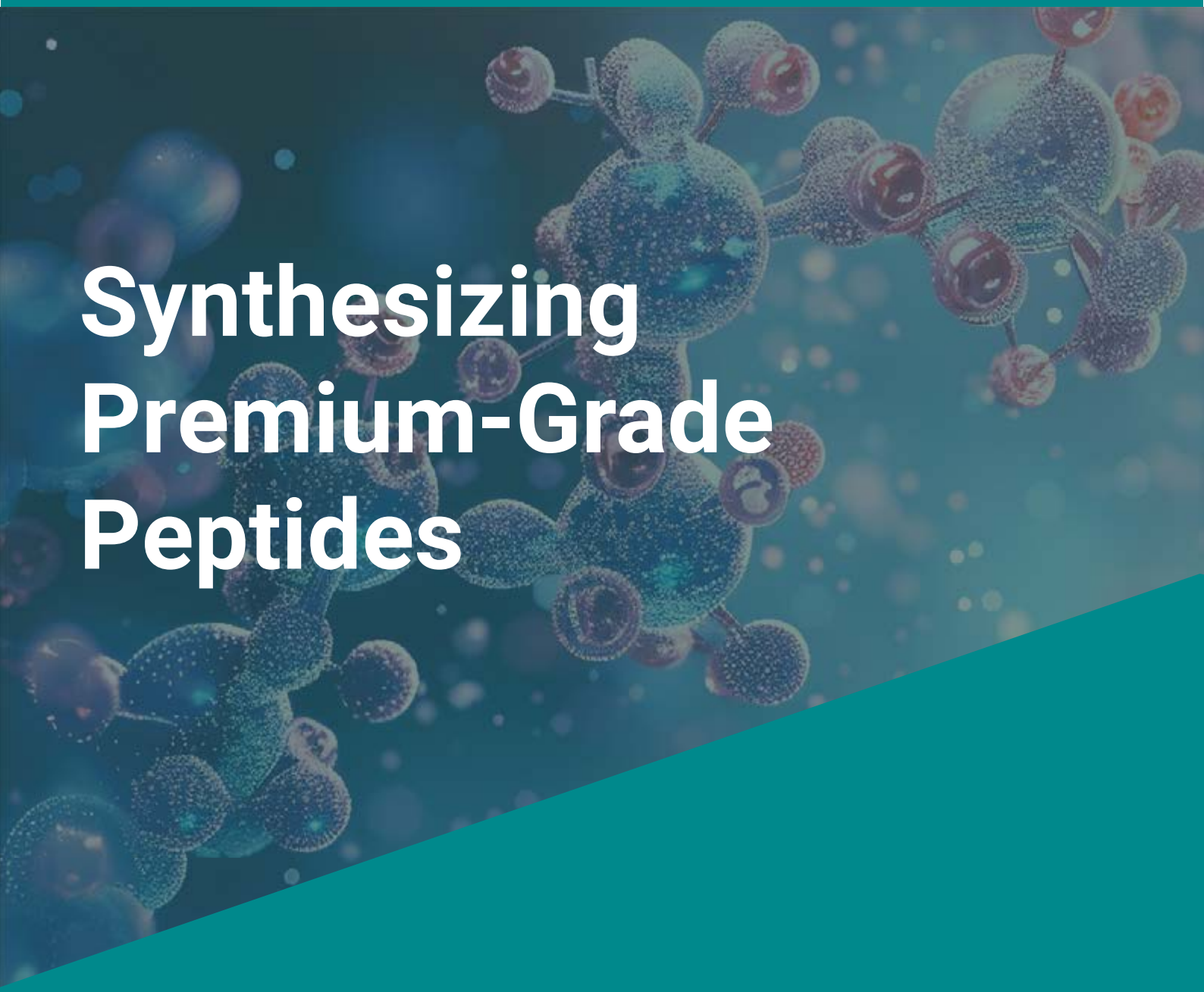
INNOVATING IMMUNOLOGY RESEARCH



Synthesizing Premium-Grade Peptides

Your guide to:

Understanding Short-Chain Peptide Products: Synthesis, Purity & Design

Peptide Pool Engineering: A Case Study Featuring Virax Biolabs ImmuneSelect EBV Peptide Pool

Engineered, chemically synthesized peptides are essential tools across the biopharmaceutical and life science research sectors. Yet, their careful design and manufacturing requires specialist knowledge, expertise, and advanced organic chemistry techniques in order to ensure structural integrity, purity, and biological functionality.

Here, we explore the technical principles of short-chain peptide production using automated solid-phase synthesis (SPPS). We demonstrate key research applications of high-purity, premium-grade peptide pools, using our ImmuneSelect EBV Peptide Pool as a case study example for life science researchers.

Contents

Understanding Short-Chain Peptide Products: A Review of Synthesis, Purity & Concentration

Peptides are versatile molecules that can be used in a broad range of life science research, drug development and bioprocessing workflows. Their manufacture, purity grading and conformational diversity reflect their suitability in a variety of highly specific applications.[1,2,3]

Understanding peptide synthesis, purity, quality and design is the first crucial step in selecting the right reagent or active pharmaceutical ingredient (API). Considering each of these aspects enables informed decisions about the suitability of a short-chain peptide or peptide pool product and ensures the quality of the experimental design.

Synthesis methods and solid-phase considerations

Chemical synthesis (notably, the solid-phase method) is typically employed in the production of short-chain peptides fewer than 60 residues in length[4] (Figure A). This method is favoured due to its scalability and high-level process control with appropriate organic chemistry knowledge. Recombinant methods (such as gene cloning and expression in model systems) are preferred for producing longer, more structurally complex molecules that require post-translational modifications.[5,6] While recombinant protein synthesis relies heavily on the integrity of cellular transcription and translation machinery, solid-phase synthesis (SPPS) enables direct and sequence-defined control of peptide assembly. Peptide sequences for SPPS can be guided by digital input files (such as .XLSX or .CSV), including those derived from peer-reviewed data repositories. As a result, SPPS-synthesized peptides are well-suited for use in research applications where a high degree of sequence specificity is required. Protocols such as the “tea bag” method and microwave-assisted synthesis approaches can also be employed to adjust the overarching reaction parameters, and the number of reaction cycles can be easily programmed into software built into the synthesizer machine.[4]

While SPPS methods are controllable using software and instrument programming

(ideal for automation), the majority of reaction optimization is achieved through tailored chemistry strategies. Resin types, protecting group, and solvent selection play a critical roles in ensuring reaction success and end product suitability.[7]

Figure A: Solid-Phase Synthesis: Linear, n+1, Sequential Amino Acid Addition.
Solid-phase peptide synthesis (SPPS) mediates the sequential addition of amino acids to a short (<80mer) peptide string, anchored by a synthetic resin.

Resin beads provide a solid support upon which peptide chain assembly can occur. A range of polymer resins are available and can be functionally differentiated by their specific reaction qualities. Different resin chemistries require different solvents in order to maximize their functionality.

To ensure these reactions proceed with high precision, the reactive sites of the amino acids must be temporarily masked using specific protecting groups. Protecting groups attach to the amine residues of each linker or peptide chain, functioning to prevent undesired side reactions during elongation. Each offers unique benefits when it comes to short-chain peptide production and can be coupled to a variety of resin types.

Understanding purity and concentration

Purity is another important consideration in peptide application suitability. In this context, purity refers to the percentage of the final product that reflects the desired, full-length amino acid sequences.

Purity levels are typically expressed as a percentage:

% purity = desired peptide/total peptide volume x 100

These percentages can be broadly grouped into three categories: over 90% pure, over 80% pure, and over 70% pure.

>90%	*Suitable for all life science applications. Essential for reliable data and reproducible research.*
>80%	*Suitable for screening and exploratory research. Some background and off-target effects may be expected.*
>70%	*Suitable for applications in which elevated background levels may not significantly impact research or downstream development.*

Note: we don't recommend using 70% purity for immune stimulation assays.

For peptide products consisting of single-sequence polymers, concentrations are easy to calculate. However, for more complex peptide pool mixtures, it is essential that the concentration of each peptide, along with the concentration of the final solution, is considered. This ensures that peptide pool products are not only accurate in their total concentration, but also contain the desired molar ratio — a critical factor for consistent biological assay performance.

Peptide solubility

Peptide solubility in water can be an important consideration for many life science research applications. Good water solubility enables peptides to be easily dissolved in biologically relevant buffers with appropriate salt conditions. In contrast, dimethyl sulfoxide (DMSO), a common non-aqueous solvent, can elicit toxicity to mammalian cells and elicit biological changes even at low concentrations. While water-based solubility issues may be inevitable in certain peptide sequences, several design considerations can help eliminate the need for DMSO.

Want to learn more about the role of SPPS in peptide pool production?

Read our latest case study on peptide pool engineering, featuring our ImmuneSelect EBV Peptide Pool pipeline.

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Peptide Pool Engineering: A Case Study Featuring Virax Biolabs' ImmuneSelect EBV Peptide Pool Technology

Authored by Dr Basusree Ghosh

Dr Ghosh is a Senior Scientist leading R&D projects at Virax Biolabs. Her strong foundation in analytical biochemistry and synthetic peptide-based therapeutics supports an extensive working knowledge of peptide pool development.

The Epstein-Barr Virus (EBV) is a human herpesvirus that is ubiquitous throughout our global population; most individuals become infected with EBV at some point in their lifetime. Although EBV infection is implicated in a variety of mild-to-severe symptomologies (ranging from asymptomatic or latent infection, to haematological, neurological and whole organ failure), its clinical and research relevance remains consistent.

In designing our ImmuneSelect EBV Peptide Pool, we considered the nuances of peptide chemistry requirements alongside the functional needs of immune research applications.

Understanding the EBV infection cycle and viral phenotypic variability

Product development began with the assessment of the global EBV research landscape and researchers' needs.

EBV primarily infects B lymphocytes; it causes their differentiation into a proliferative plasma cell state and migration into the peripheral circulatory system. As a member of the Herpesviridae family (Figure 1), it shares the hallmark ability to persist indefinitely within the body. Once its immune evasion is established, EBV can sustain a lifelong infection of the host lymphocyte pool, remaining latent yet poised for reactivation.

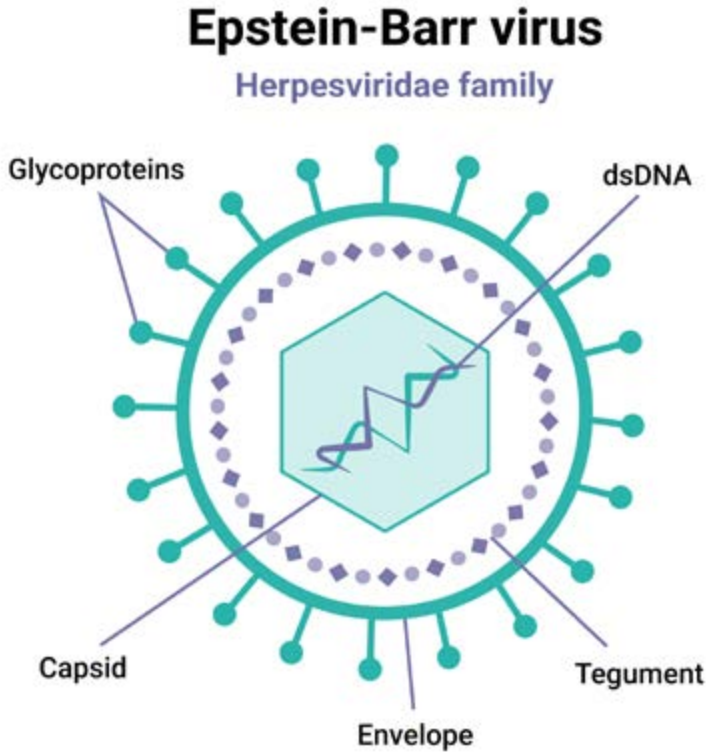

Figure 1: Structural Diagram of the Epstein-Barr Virus (EBV).
Schematic representation highlighting the key anatomical components of a mature EBV virion, a member of the Herpesviridae family.

Two major, genetically distinct EBV strains currently exist in our population; Type 1 is the globally dominant variant, while Type 2 is known to circulate in distinct locations. Both can be distinguished by genetic variability in their EBV nuclear antigen (EBNA) genes - an important consideration in epitope selection and peptide pool design.

Designing an EBV peptide pool that meets the needs of diverse immunology researchers

During the design phase of our ImmuneSelect EBV Peptide Pool, the diverse global EBV research community was considered. While the EBV Type 1 variant is globally dominant, it was important to ensure that Type 2 and emerging variant research were not overlooked. We also aimed to provide coverage of the complete infection lifecycle to maximize our product utility across diverse immunology, virology, and cancer research applications. Some of the key considerations made were:

1. Peptides were selected from highly conserved regions of the EBV proteome to mitigate the impact of mutations or variant-specific effects.

2. Peptide length was selected to maintain aqueous solubility of the final product, avoiding the need for DMSO as a solvent.

3. The number of peptides in our peptide pool mix was optimized generate robust immune responses, as verified by ELISpot.

4. Non-overlapping peptide strings from different proteins were selected with the intent of supporting T cell stimulation and broad application.

5. By selecting strings between 8 and 21 amino acids, we accounted for epitopes presented on both major histocompatibility complex (MHC) class I and class II molecules.

Application Tip:

The same epitope selection work-flow can be applied to any viral or bacterial research target.

You can also apply the same principles to immunotherapeutic research applications.

Building an ImmuneSelect peptide pool

We employ a variety of bioinformatic and peptide chemistry techniques to build our ImmuneSelect EBV Peptide Pools. Our work-flow integrates open-source, online platforms informed by over 26,000 references to peer-reviewed publications, with established, gold-standard protocols, ensuring that scientific evidence leads our product design and manufacture.[2] We follow industry best practice procedures in solid-phase peptide synthesis (SPPS), high-performance liquid chromatography

(HPLC) and liquid-chromatography-mass spectrometry (LC-MS). Broadly, our workflow can be categorized into four distinct steps (Figure 2).

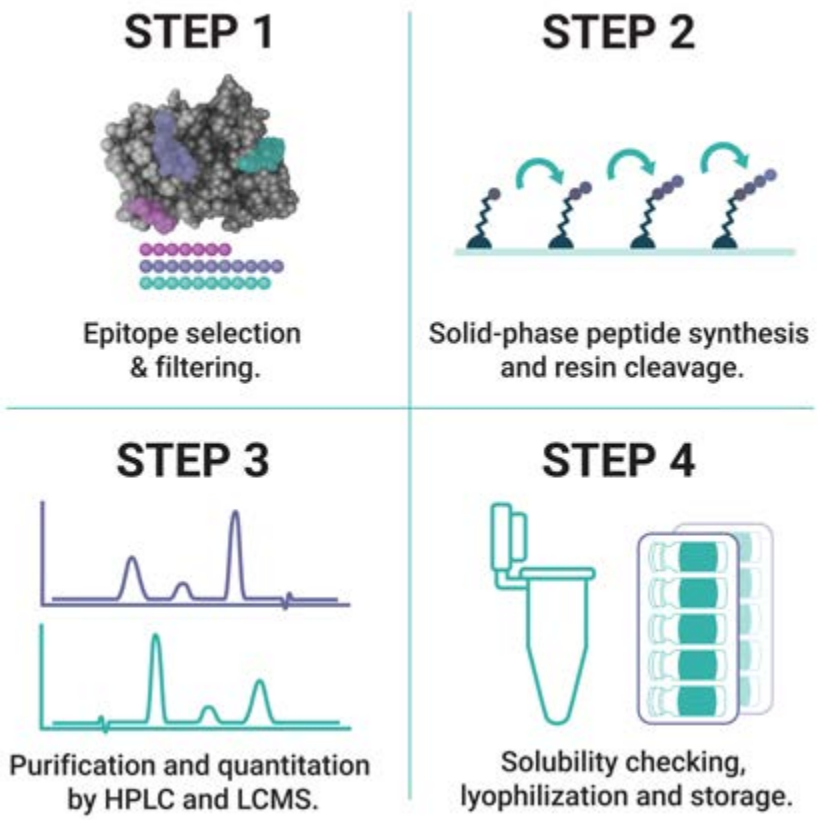

Figure 2: General Workflow for Synthetic Peptide Epitope Production.
Overview of the four-step pipeline utilized to generate custom peptide antigens.

Step 1: Epitope selection and filtering

Epitope selection and filtering are critical to the development of an effective peptide pool; hence, we consider both the complexity of the viral or bacterial target and the availability of validated sequence research as part of this initial workflow step. While EBV is a relatively well-known, well-researched pathogen, other targets may have less well-informed sequence data available. For EBV, we begin our epitope selection and filtering process with peer-reviewed, published sequence data that can be found in public repositories such as the National Institute of Allergy and Infectious Disease (NIAID)-funded Immune Epitope Database (IEDB).

We search the database according to the parameters and expectations outlined during product design, selecting linear peptides that stimulate human T cells (CD4+

and CD8+) through both major histocompatibility complex (MHC) class I and class II pathways. Peptides are further filtered according to best practices in SPPS, removing peptide strings that are likely to exhibit poor solubility issues or be susceptible to conformational folding.

Immunology researcher requirements are also considered, ensuring that selected peptides are suitable for cellular uptake and exhibit a low risk of cytotoxicity. The final set of 44 peptides (Figure 3) is compiled into a .CSV or XLSX file, ready for upload onto our automated peptide synthesizer. Protein source details can be found in Table 1, or via the ImmuneSelect EBV Peptide Pool page on our website.

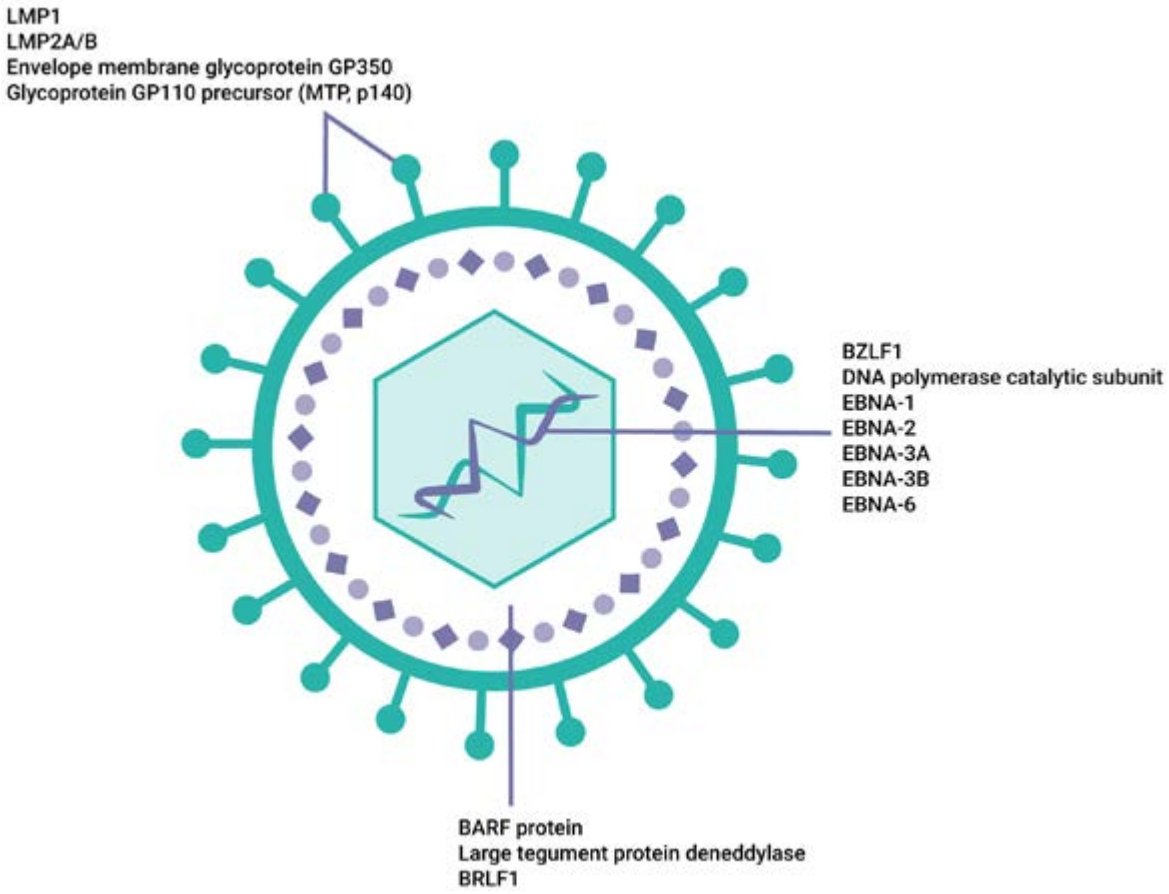

Figure 3: Localization of Epstein-Barr Virus Source Proteins Selected for the Peptide Pool.
Schematic map of EBV proteins used to derive the synthetic peptide pool, classified by their structural compartment within the virion: envelope, tegument, and nucleus.

Similar details for each of our peptide pool products are included on their respective web pages. We also supply these detailed reports to custom peptide orders upon request.

Table 1: The 44 Linear Epitopes That Make up the ImmuneSelect EBV Peptide Pool.

Index	Source Protein	MHC-Class	Sequence
1	BALFZ1	I	ARYAYYLQF
2	BARF protein	I	FLGERVTLT
3	BARF protein	I	TLTSYWRRV
4	BARF protein	II	HRSANTFFLVVTAANK
5	BARF protein	II	LGERVTLTSYWRRVS
6	BARF protein	II	PFRGFFDIHRSANTF
7	BRLF1	I	RVRAYTYSK
8	BZLF1	I	SENDRLRLL
9	BZLF1	I	RAKFKQLL
10	DNA polymerase catalytic subunit	II	TGGVYHFVKKHVHES
11	EBNA-1	II	YFMVFLQTHIFAE
12	EBNA-1	II	DGGRRKKGGWFGKHR
13	EBNA-1	II	LRALLARSHVERTTD
14	EBNA-1	II	KMVFLQTHIFAEVLKD
15	EBNA-1	II	THIFAEVLKD
16	EBNA-1	II	KTSLYNLRRGTALA
17	EBNA-1	II	RALLARSHVERTTDE
18	EBNA-1	II	KFENIAEGLRALLAR
19	EBNA-1	II	VYGGSKTSLYNLRRGTALAI
20	EBNA-1	II	NPKFENIAEGLRALLARSHV
21	EBNA-1	II	ENIAEGLRVLLARSHVERTT
22	EBNA-2	II	MANYIVRQSRGDRGL
23	EBNA-3A	I	FLRGRAYGL
24	EBNA-3A	I	QAKWRLQTL
25	EBNA-3A	I	RLRAEAQVK
26	EBNA-3A	I	SVRDRLARL
27	EBNA-3B	I	IVTDFSVIK
28	EBNA-6	II	FWEMRAGREITQ
29	Glycoprotein GP110 precursor	I	FLDKGTYTL
30	Glycoprotein GP110 precursor	II	PGWLIWTYRTRTTVNR
31	Large tegument protein deneddylase	II	FARQAVWLRE
32	LMP1	I	YLLEMLWRL
33	LMP1	II	LWRLGATIWQLLAFFK
34	LMP1	II	SGHESDSNSNEGRHHK
35	LMP1	II	TDGGGGHSHDSGHGG
36	LMP1	II	TDDSGHESDSNSNEGRHR
37	LMP1	II	KGGSILQTNFKSLSSTEF
38	LMP2	II	KVLVMLVLLILAYRRRWRRLT
39	LMP2	I	RRRWRRLTV
40	Major capsid protein	II	SMYRKIYGELIALEQALMRL
41	p140	II	ARELALVGILLGNGER
42	p140	II	LGAIKHQALDTVRYD
43	p140	II	NHLVLFDNALRKYDS
44	p140	II	RQERARELALVGILL

Step 2: Solid-phase peptide synthesis (SPPS), cleavage and precipitation

SPPS is a well-established, reliable method for producing high-purity peptide pools, offering a consistent alternative to biological approaches, such as cell-derived or recombinant products, which can exhibit excessive biological variability or lysosomal degradation.

SPPS methods are also compatible with scalable and automated production workflows (Figure 4). We work with the Symphony X automated peptide synthesizer from Gyros Protein Technologies, featuring 24 independent reaction vessels, which allow the application of different protocols and reaction times. This also enables rapid synthesis of custom and stock peptide products.

While the Symphony X can be guided by carefully programmed inputs, reaction performance depends on careful chemical optimisation. In each of our Symphony X reaction vessels, we use a pre-loaded Wang resin, coupled with Fmoc chemistry; this reflects SPPS best practices by pairing optimal amino acid loading with gentle reaction conditions. N,N-Dimethylformamide (DMF) is used, with minimum water content (less than 0.03%) as a polar, aprotic solvent; piperidine as a gentle deprotecting agent; N-ethyl diisopropylamine (DIPEA) as the base; and HBTU/HCTU/TBTU/PyBOP activators used according to synthesis needs. After every run, system cleanliness is ensured with a dichloromethane (DCM) backflush – this further reduces the risk of batch effects or artefact contamination, and guarantees the highest purity of subsequent synthesis runs.

The 44 peptides in the ImmuneSelect EBV Peptide Pool are variable in sequence length; as a result, some peptides require more SPPS cycles than others for synthesis to be completed. Once enough cycles have occurred to synthesize the entirety of the longest peptide chain, the SPPS reaction is considered complete, and the new, resin-attached peptides are left to dry. The crude products are then collected from their respective reaction vessels and each transferred to a separate desalting column for cleavage from the resin beads.

Most solid-phase synthesis cleavage protocols require treatment with a strong acid cocktail to simultaneously detach the synthesized peptide from the resin and remove all remaining protecting groups from the amino acid side chains. However, this chemical treatment can sometimes lead to unwanted side reactions or damage

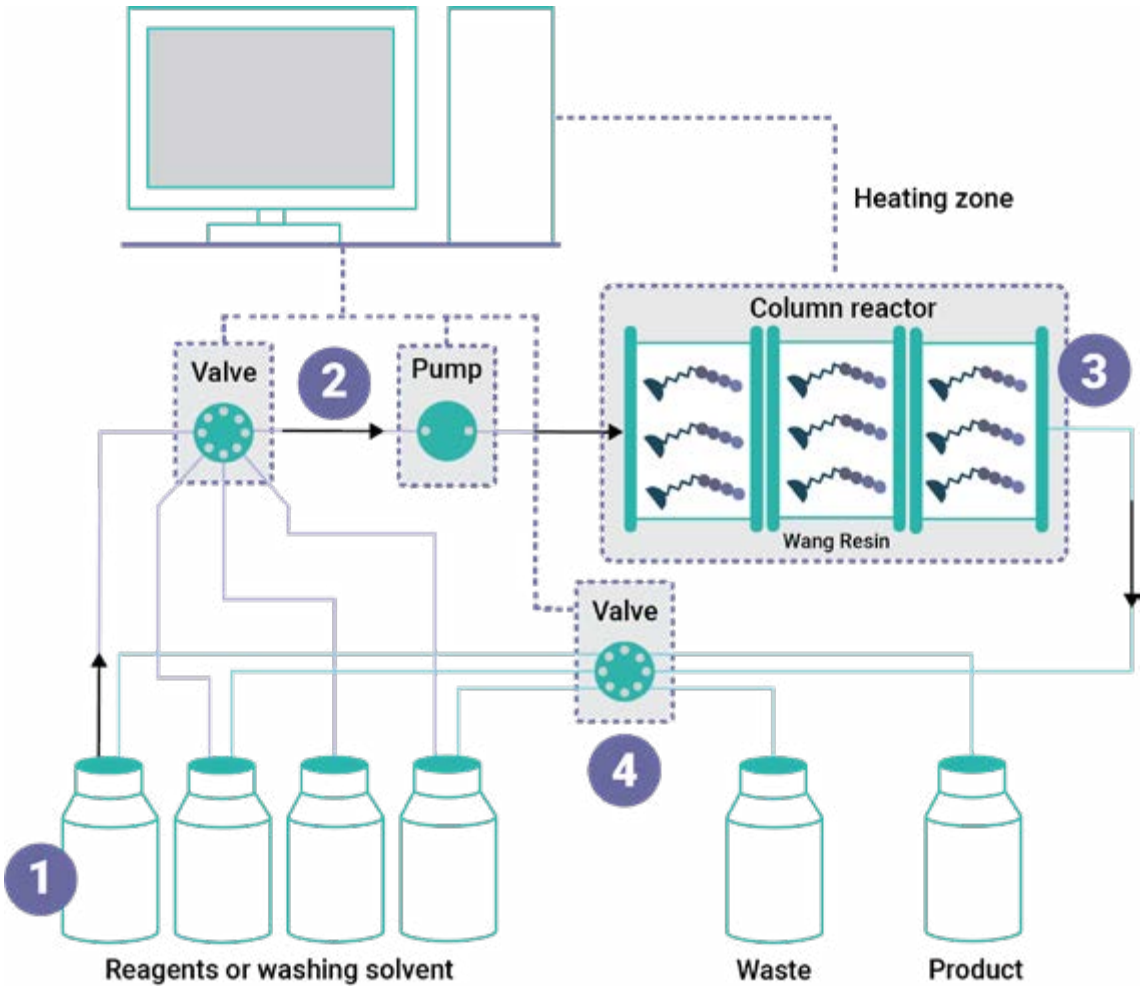

Figure 4: Automated Solid-Phase Peptide Synthesis Workflow.

Schematic representation of the integrated fluidics, automated control, and reaction architecture for peptide elongation: (1) Reagent Storage: selection of specific amino acid monomers, coupling agents, or deprotection reagents from automated fluid reservoirs. (2) Fluidic Delivery: computer-controlled automated reagent transport. (3) Column Reactor: temperature-controlled synthesis zone containing functionalized Wang resin for iterative cycles of deprotection, washing, and coupling. (4) Product Routing: fluid stream is directed to waste containment or final product collection.

to sensitive amino acid residues, necessitating the addition of "scavengers" to the cleavage cocktail to protect these fragile components. Our cleavage protocol ensures robust cleavage while maintaining product protection.

Following cleavage, the mixture is poured into chilled diethylether, filtering out the resin and precipitating the peptides. Cleavage reactions are quenched at -20°C to minimize the risk of modifications and support efficient precipitation.

Step 3: Purity and concentration analyses

Unless otherwise stated, our ImmuneSelect peptides are created to a premium >90% purity target, with rigorous quality control performed on every batch (Figure 5).

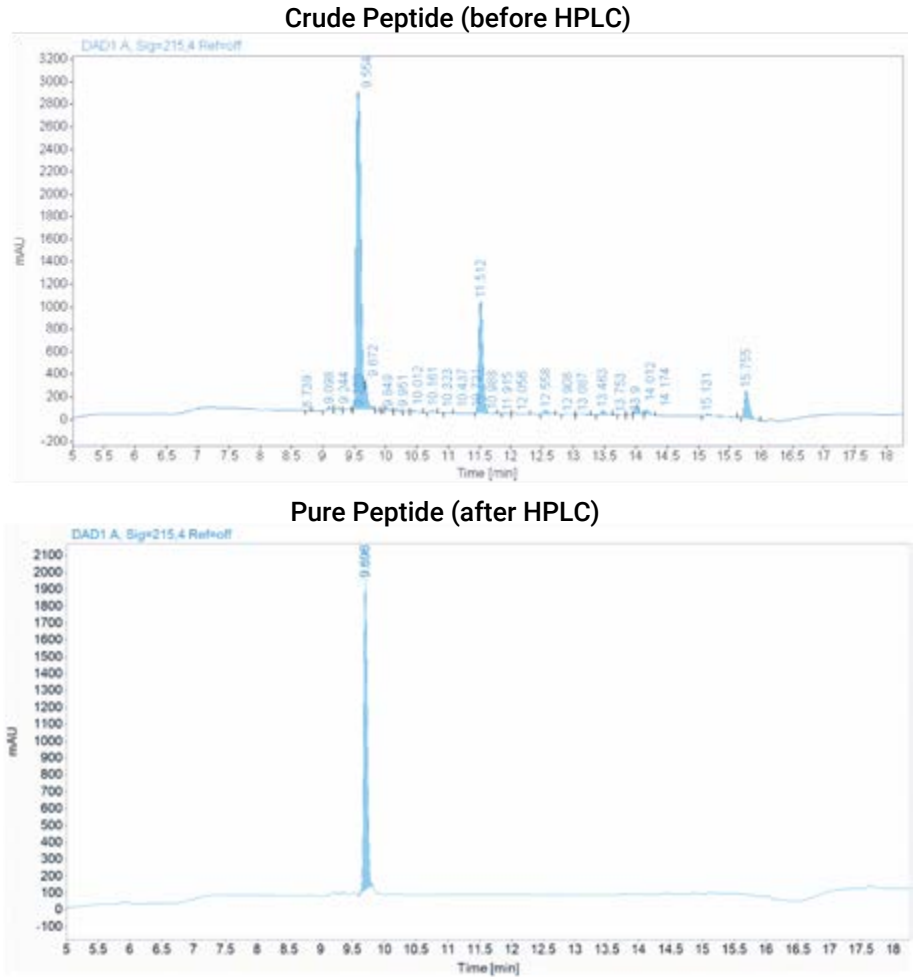

Figure 5: HPLC Chromatograms Demonstrating Purity Verification of ImmuneSelect Peptides.

Crude Peptide (top): Profile immediately post-cleavage, showing a complex mixture containing deletion sequences, truncated fragments, and protecting group byproducts alongside the main target peak. Pure Peptide (bottom): Profile following HPLC purification, confirming successful removal of chemical contaminants and attainment of the premium >90% purity target, indicated by the single, sharp target peak.

LC-MS is a key analytical technology for peptide pool purification; allowing highly-specific, mass-directed (m/z ratio) fractionation of crude products, ensuring only correctly synthesized sequences are included in final formulations. LC-MS is particularly useful in identifying and removing contaminant protein fragments - as well as system reagents - which may exhibit similar physical, hydrophobic properties.

Because closely-related side products like truncated sequences or deletion fragments have nearly identical chemical properties, they exit the column at almost the same time, making minor retention time differences insufficient for a reliable separation. We conduct LC-MS analysis both before and after HPLC purification, ensuring that potential contaminants are removed, and the desired peptide products remain structurally intact.

Unlike many analytical HPLC protocols (which focus on reducing sample loss), our purification workflows are optimized for fragment retention at scale. Our preparatory reverse-phase HPLC (prep RP-HPLC) methods generate purified peptides at substantial concentration and volume - rather than performing separation only for quality assessment. This capability is a key benefit of our custom services for researchers seeking to scale up peptide production while minimizing the risk of costly in-house preparatory equipment.

Application Tip:

For researchers looking to expedite peptide pool development without the cost-of-ownership associated with HPLC/LC-MS, we are on hand to act as your trusted research and development partner. Get in touch to speak to one of our Virax Biolabs experts.

Step 4: Pooling, lyophilization and solubility checking

Prior to peptide pooling, the concentration of each pure fragment is checked by UV-Vis spectroscopy. Peptide quantitation is performed using Waddel's method, an established approach that accounts for the absorbance variability of aromatic amino acids. Finally, pure peptide concentrations are standardized to 7nmol, suitable for *in vitro* applications such as ELISpot, FluoroSpot and other cell-based assays. Lyophilization transforms the pooled, aqueous peptides into a stable, dry powder, ideal for long-term storage and transport. This process involves controlled freezing,

sublimation and gentle desorption to remove water, preserving the structural and chemical integrity of the product.

The final stage in ImmuneSelect EBV Peptide Pool production is solubility verification. ImmuneSelect peptides are designed for high aqueous solubility, ensuring they can be used without the need for harmful organic solvents. Since even trace amounts of DMSO (<1%) can disrupt T cell metabolism[3], the excellent aqueous solubility of our peptides allows for safe, direct application in the sensitive cell-based assays widely used by EBV researchers worldwide.

Figure 7: ImmuneSelect Premium Grade Peptide Pool.

To shop our full range of pre-designed ImmuneSelect peptide pools and custom peptide pool production services, visit research.viraxbiolabs.com or contact one of our experts.

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