Automated High-Throughput Peptide Purification

Parallel purification of 192 peptides in plate-based format using Biotage® PeptiPEC-96 High-Throughput Kits

Introduction

High-throughput library synthesis is typically required in the early stages of the drug discovery process. Despite the many advances in automated peptide synthesis technologies, the limited throughput of conventional purification technologies creates a major bottleneck in producing peptide libraries. Using reversed-phase high-pressure liquid chromatography (RP-HPLC) to purify hundreds of compounds sequentially comes with substantial cost, not only in time but also solvent consumption. Due to the throughput limitations of sequential purification, crude peptides are being evaluated in assays, which increases the risk of false-positive or false-negative results and thus decreases the reliability of assay results.

The Biotage® PeptiPEC-96 High-Throughput Kit, is based on the PurePep® EasyClean (PEC™) technology from Gyros Protein Technologies, using a novel reductively cleavable linker molecule (PEC-Linker RC+) and activated filter materials (aldehyde modified agarose beads), in a 96-well plate format.

PEC™ catch-and-release technology has been designed for the purification of chemically synthesized peptides and the 19-mer peptides P1 and P2 in Figure 1, show some examples of the purities achieved after PEC purification.

Herein, we demonstrate the use of Biotage® PeptiPEC-96 High-Throughput Kits and Biotage® Extrahera™-Peptide workstation to enable the automated purification of a peptide library containing 192 SARS-CoV-2 epitopes for use in a T-cell response assay.

Experimental

Synthesis

All peptides were prepared by Fmoc solid-phase peptide synthesis. 192 peptides were synthesized in parallel at 10 µmol scale on Ramage Amide AM resin. Fmoc deprotections were performed 2x for 5 min for the first 5 cycles and 2x for 6 min thereafter with 0.77 v% formic acid in 20 v% piperidine in DMF (0.1 eq. formic acid and 1 eq. piperidine). Single peptide couplings (1 x 45 min.) were performed using 4 eq. of Fmoc amino acid (0.5 M), 6 eq. DIC (1.5 M in DMF) and 4 eq. Oxyma (1 M in DMF). From the 6th residue, double couplings (2 x 45 min.) were performed using 4 eq. of Fmoc amino acid (0.5 M), 3.9 eq. HATU (0.5 M in DMF) and 7.8 eq. DIPEA (50% v/v in NMP) for the first coupling and the second coupling using DIC/Oxyma as for single couplings. Capping was performed after every coupling step using 4 M pyridine in DMF and 4 M Ac2O in DMF reacting for 5 min. The reductively cleavable PEC-Linker RC+ was coupled to the full-length target peptides as the last building block using 4 eq. in DMF, DIPEA (6 eq.) and Oxyma (6 eq.) for 4 h. TFA cleavage was performed for 2 h using TFA/H2O/EDT/PhSMe/TIS (83:5:5:5:2, 0.5 mL cleavage cocktail per 10 µmol peptide). The crude linker-modified peptides were collected, precipitated, centrifuged, and dried in a 96- well plate.

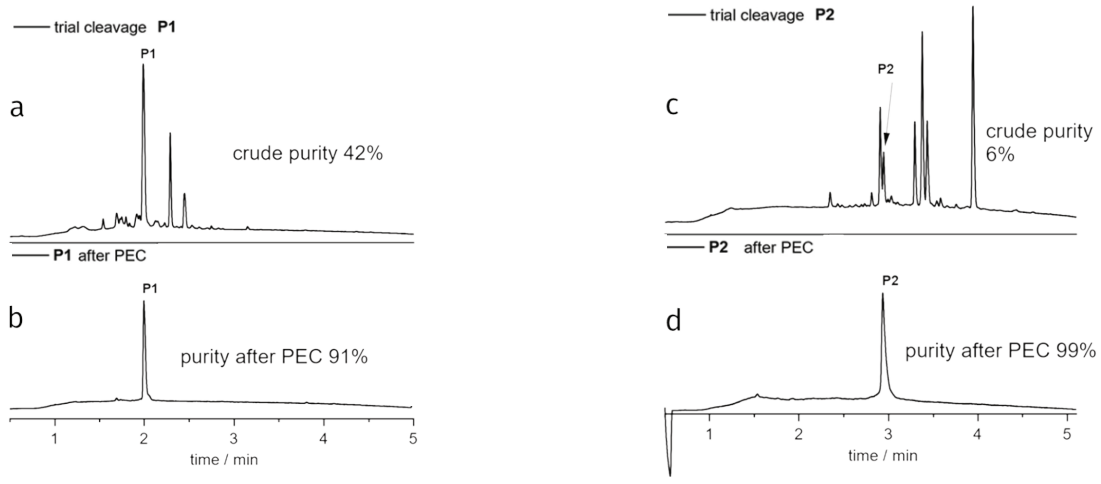


Figure 1. PEC Purification Data Examples (19-mer peptides P1 & P2); a) Analytical HPLC crude peptide P1; b) Analytical HPLC PEC purified peptide P1; c) Analytical HPLC crude peptide P2; d) Analytical HPLC PEC purified peptide P2.

Purification

Crude peptides were dissolved with 500 µl HFIP/well and shaken for ≥ 1 h. The peptide sample plate was loaded onto the Biotage® Extrahera™-Peptide workstation where immobilization of the PEC-Linker-tagged target peptides, washing, and final release was performed automatically.

The PEC-grade peptides were collected in a deep well plate and directly lyophilized. The PEC purified peptides were analyzed and weighed. UV-purity was determined from the final UV-chromatograms by peak-integration at a wavelength of 210 nm.

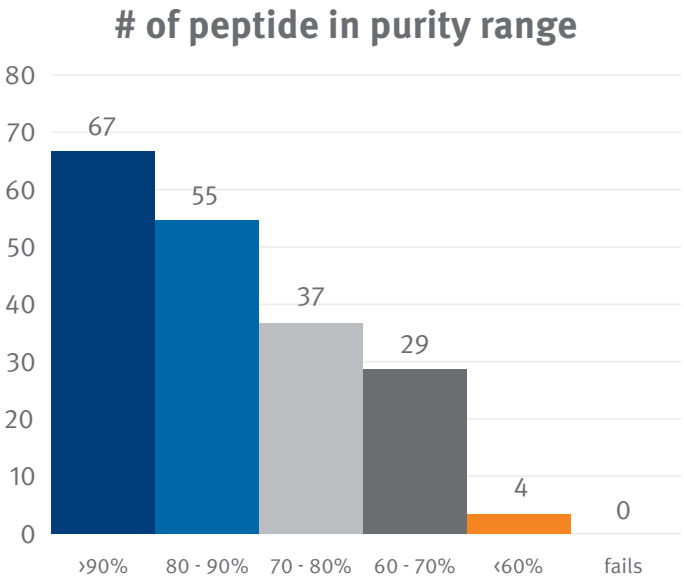
Results & Discussion

A peptide library of 192 SARS-CoV-2 epitopes covering the spike protein of SARS-Cov2 omicron variant was synthesized with C-terminal amides and purified using Biotage® PeptiPEC-96 High-Throughput Kits and Biotage® Extrahera™ workstation in two batches. The optimized, automated strategy enabled purification of 96 peptides simultaneously with minimal manual intervention, requiring ~13.5 hours of total time and approximately 1 L of total solvent (aqueous and organic mixtures) per purification.

The peptide library was used to investigate how the immune system develops over time after undergoing omicron infection in vaccinated health-care workers with and without previous SARS-CoV-2 infection. The presence of peptidic impurities significantly compromises the integrity of these data results, therefore requiring some level of purification prior to analysis. Traditional, sequential HPLC is a less-than-ideal purification strategy in this case due to the time constraints placed on synthesis, purification, and ultimately assay evaluation.

- **Objective:**
  - Prepare 192 omicron-derived peptides with >70% purity and in >0.5 mg quantity for use in T-cell response assays.
- **Outcome:**
  - 188 peptides met crude purity requirement on first attempt.
  - 182 peptides met minimum quantity requirement on first attempt.
  - Delivered library to client for assay evaluation in less than 2 weeks
- **Library details:**
  - Peptides were 15-18 (standard) amino acids in length.
  - 90/192 sequences contained Cys or Met.
  - Average peptide library purity: ~83%.
  - Average peptide quantity delivered: 3.4 mg.

Figure 2 below provides an overview of the purity analysis and quantities obtained for the peptide library.



Final purity range	Complete Set # of peptides
>90%	67
80-90%	55
70-80%	37
60-70%	29
<60%	4
fails	0
SUM	**192**
Mean purity	**83%**
Ordered final amount:	**# of peptides**
> 0.5 mg	96
< 0.5 mg	0
> 1 mg	86
< 1 mg	10
Mean weight [mg]	**3.4**

Peptides below target purity	4
Peptides below target amount	10
Sum	14
Percentage of peptide numbers	7%
Success rate	93%

Figure 2. PEC Purified Library Purity Overview

Table 1 below shows some representative examples of the purity data for 10 peptide sequences in the peptide library.

Table 1. Representative Peptide Purity Data for 10 Peptides.

PEC-ID	Sequence	Length	Mol Weight [g/mol]	Exact Mass [g/mol]	m/z (calc.)	m/z (obs.)	UV-purity (210 mm)	Determined Weight [mg]
SO362_002	FHAIHVSGTNGTKRFDNP	18	1998.194	1995.924	666.4 / 999.5	666.9	84.9%	6.25
SO362_014	SVLYNSASFSTFKCYGVS	18	1960.191	1957.846	653.7 / 980.5	1019.8	86.1%	3.92
SO362_023	FNCYFPLQSYGFQPTNGV	18	2082.321	2079.876	694.4 / 1'041.5	1041.2	96.6%	5.54
SO362_042	KFNGLTVLPPLLTDEMIA	18	1972.375	1970.012	657.7 / 986.6	986.6	93.9%	2.92
SO362_044	DVVNQNAQALNTLVKQLS	18	1955.203	1952.983	652.0 / 978.0	977.8	100.0%	2.40
SO362_053	NDPFLDHKNNKSWME	15	1875.049	1872.796	625.3 / 938.0	937.7	77.1%	3.98
SO362_114	VFKNIDGYFKIYSKHTPI	18	2170.545	2168.103	723.8 / 1'085.7	724.2	100.0%	5.05
SO362_080	EYVNNSYECDIPIGAGIC	18	1960.162	1957.777	653.7 / 980.5	980.3	67.8%	3.34
SO362_157	KRSFIEDLLFNKVTLADA	18	2080.417	2078.071	693.7 / 1'040.6	1040.6	92.1%	5.06
SO362_161	PPLLTDEMIAQYTSALLA	18	1947.277	1944.938	649.4 / 974.1	974.1	89.1%	1.42

Upon receipt and analysis, it was shown that a significant reduction in T-cell activity was observed for persons that have not been exposed/infected to omicron compared to previous variants of Covid-19 (data not shown). This information suggests that the peptides supplied, purified using Biotage® PeptiPEC-96 High-throughput kits, were of sufficient purity (›83% average) for the T-cell response assay. Automating the purification with an optimized, one-method-fits-all protocol enabled rapid delivery of the peptide library in a timeline difficult, if not impossible to meet using standard HPLC methodologies.

Conclusion

Biotage® PeptiPEC-96 High-Throughput kits, when coupled with Biotage® Extrahera™ workstation enabled rapid delivery of an assay-ready peptide library containing 192 distinct peptide sequences. The development of an optimized, automated, one-size-fits-all purification workflow for plate-based peptide libraries provides a fast and environmentally sustainable solution for high-throughput parallel peptide purification that can improve assay reliability by strategically removing peptide-related impurities.

Acknowledgements

P1 and P2 PEC purity data reproduced by kind permission of Gyros Protein Technologies.

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