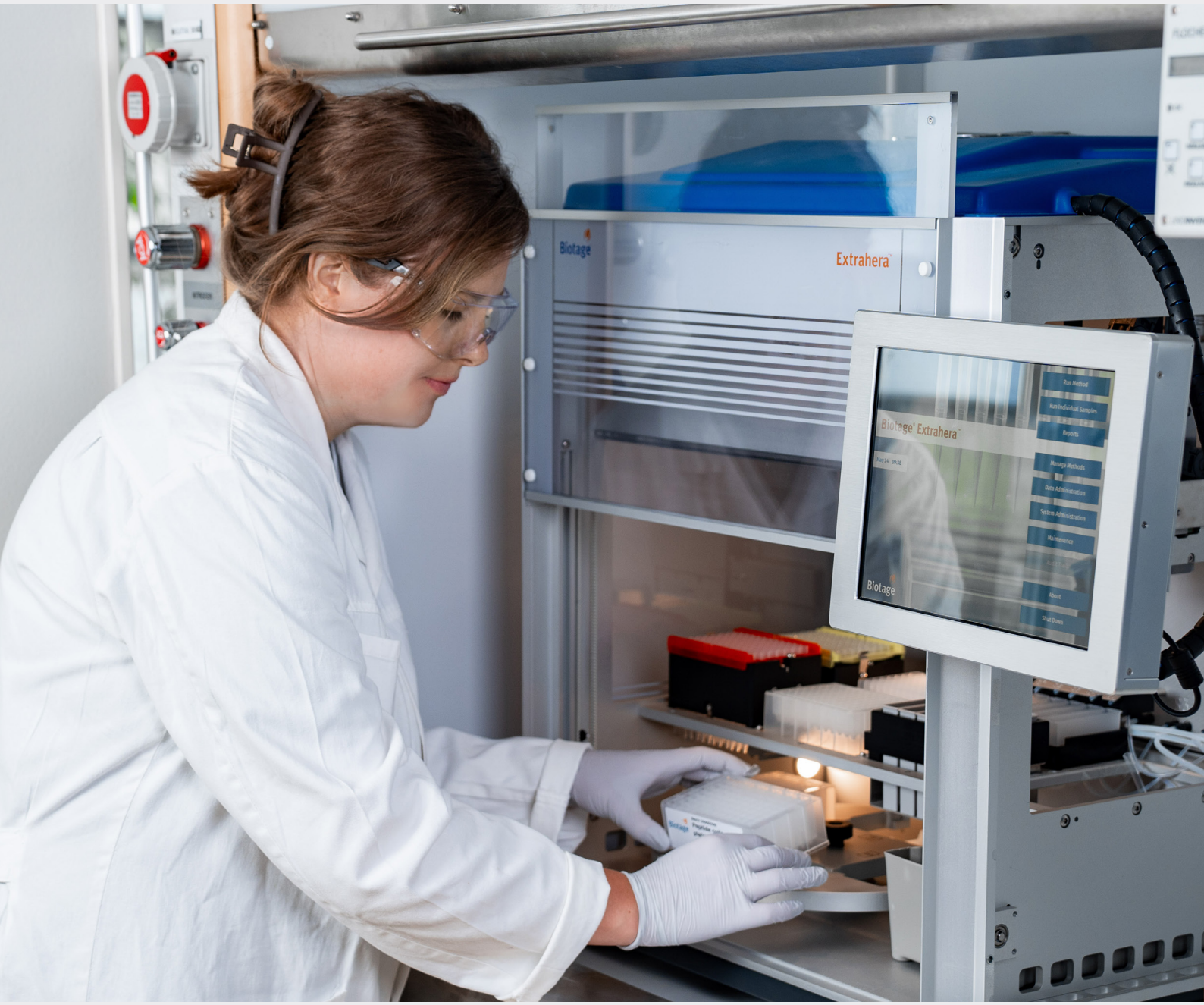
Automated High-Throughput Parallel Peptide Purification

Unparalleled Peptide Drug Discovery Workflow Solutions



Biotage®

Automated High-Throughput Parallel Peptide Purification

Peptides as a therapeutic modality have surged to the forefront of many discovery efforts, resulting in numerous globally approved drugs and many more currently undergoing clinical development. New technologies have expanded peptide therapeutics beyond metabolic disorders, with many programs now addressing clinical needs against various cancers, immunological, and cardiovascular conditions.

In the initial phases of drug discovery, high-throughput library synthesis is essential for preliminary screening, validation, and preliminary lead optimization. However, purification is a major bottleneck in producing peptide libraries. Reversed-phase high-performance liquid chromatography (RP-HPLC) is the most common method for peptide purification, but it entails significant costs, in terms of both time and solvent usage. Due to the costs and throughput limitations of sequential purification, crude peptides are being evaluated in assays, which increases the risk of false-positive or false-negative results, thereby affecting the assay outcomes.

To bridge the efficiency gap between peptide library synthesis and purification, Biotage offers two plate-based, automated parallel purification solutions, providing a range of purities suitable for various testing outcomes.

Biotage® PeptiRen-96 utilizes reversed-phase C18 functionalized media for solid-phase extraction (SPE), enhancing the purity and enrichment of crude peptide libraries. This makes it ideal for preliminary screening applications that often rely on crude samples.

Alternatively, Biotage® PeptiPEC employs a novel catch-and-release methodology for purifying chemically synthesized peptides, resulting in improved reliability of validation assays and structure-activity relationship (SAR) studies.

When used with the Biotage® Extrahera™ workstation, both solutions offer several benefits compared to HPLC, including simpler, faster, greener and more cost-effective parallel peptide purification processes, significantly reducing time and solvent usage.

- **Simpler** – Simple pre-optimized protocols to improve peptide purity
- **Faster** – Reduced purification time by parallel operation; up to 96 peptides at a time
- **Greener** – Reduced use of organic solvents
- **Cost-effective** –Increased workflow efficiency and productivity

Optimizing Time and Solvent Use in Peptide Purification

Purifying 96 peptides using Biotage® PeptiPEC-96 or Biotage® PeptiRen-96 plates with the Biotage® Extrahera workstation, reduces solvent consumption by up to 98% and speeds up the purification process by up to 98% with Biotage® PeptiRen-96 and up to 75% with Biotage® PeptiPEC-96, compared to sequential processing with RP-HPLC.

Purification method	Time (h)	Solvent consumption (L)	
Sequential processing RP-HPLC (35 min run)	56	50	
Biotage® PeptiRen-96 with Biotage® Extrahera™	1.55	1	
Manual PEC high-throughput kit	37.25	0.87	
Biotage® PeptiPEC-96 with Biotage® Extrahera™	13.50	1	

Choosing the Ideal Solution: Comparing Biotage® PeptiRen-96 and Biotage® PeptiPEC-96 for Your Peptide Purification Needs

	Biotage® PeptiRen-96	Biotage® PeptiPEC-96
Technique	C18-based Solid-phase extraction (SPE)	Catch-and-release methodology based on a novel linker molecule and activated filter material
Type of peptides	Any, for example: linear, side-chain cyclized and head-to-tail cyclic peptides	Requires a free amino terminus, for example: linear, side-chain cyclized peptides
Loading Capacity	1-15 mg crude sample per well	10 µmol sample per well
Purity (UV) requirement	>60% average purity -Removes disruptive by-products: salts, side chain protecting groups	>80% average purity -Removes peptidic impurities: truncation and deletion sequences
Method run time for 96 peptides	~1.55 h	~13.5 h
Synthesis modifications	None	Capping required after each coupling reaction
Method development	Optional, based on sample properties	None

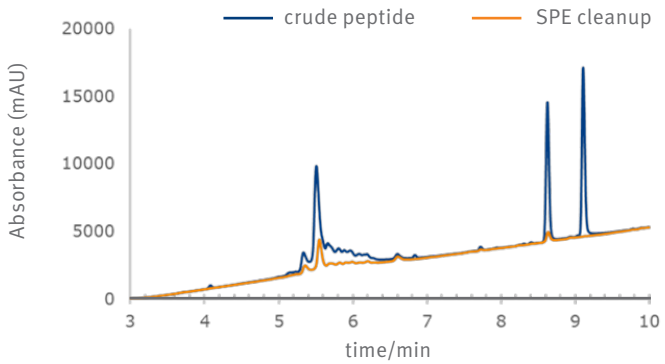


Figure 1. Crude peptide mixture cleaned up using Biotage® PeptiRen-96. The major peak (~5.5 min) is a 37-mer peptide, other significant peaks are residual protecting groups.

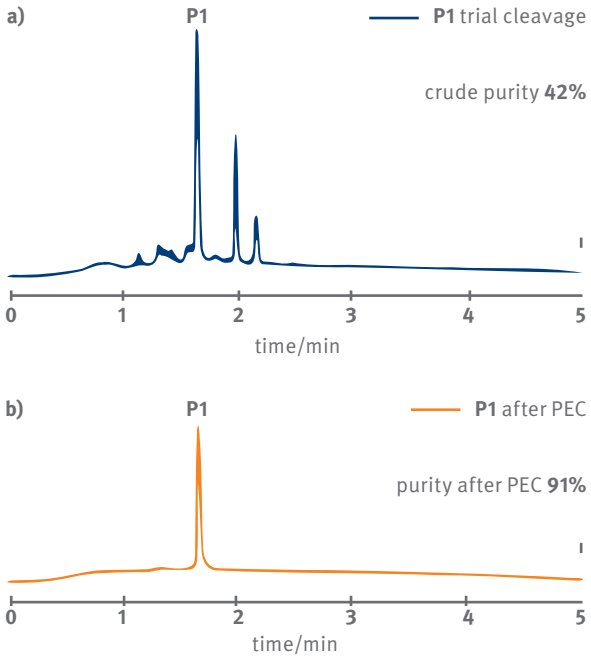
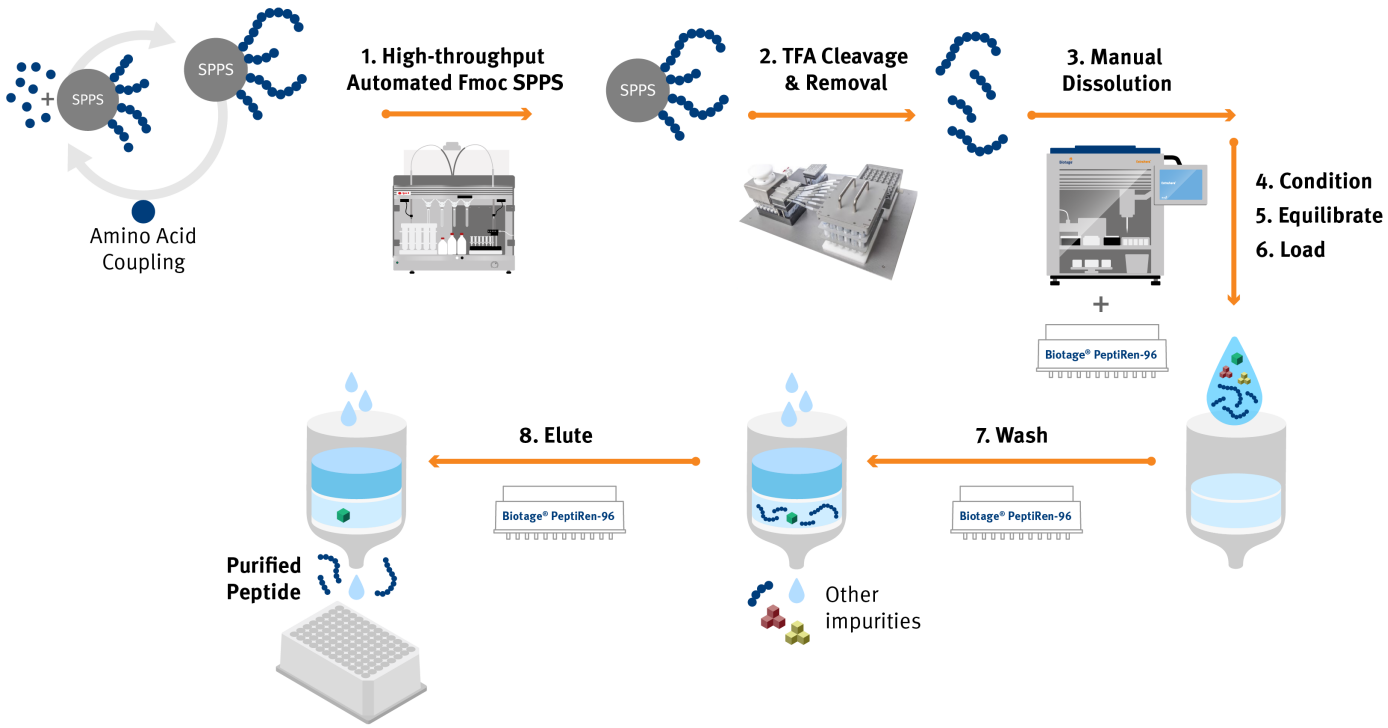


Figure 2. Analytical HPLC chromatograms showing a) P1 19-mer crude peptide and b) P1 19-mer peptide after PEC purification.

How it works: Peptide Purification Process with Biotage® PeptiRen-96 and Biotage® Extrahera



Solid-Phase Extraction for Peptide Purification

Solid-phase extraction (SPE) is a straightforward and recognized technique for extracting compounds from a complex sample mixture, and also well-suited for high-throughput workflows. It enables predictable separation of peptides from impurities, delivering peptides suitable for biological assays and simplifying peptide drug discovery workflows.

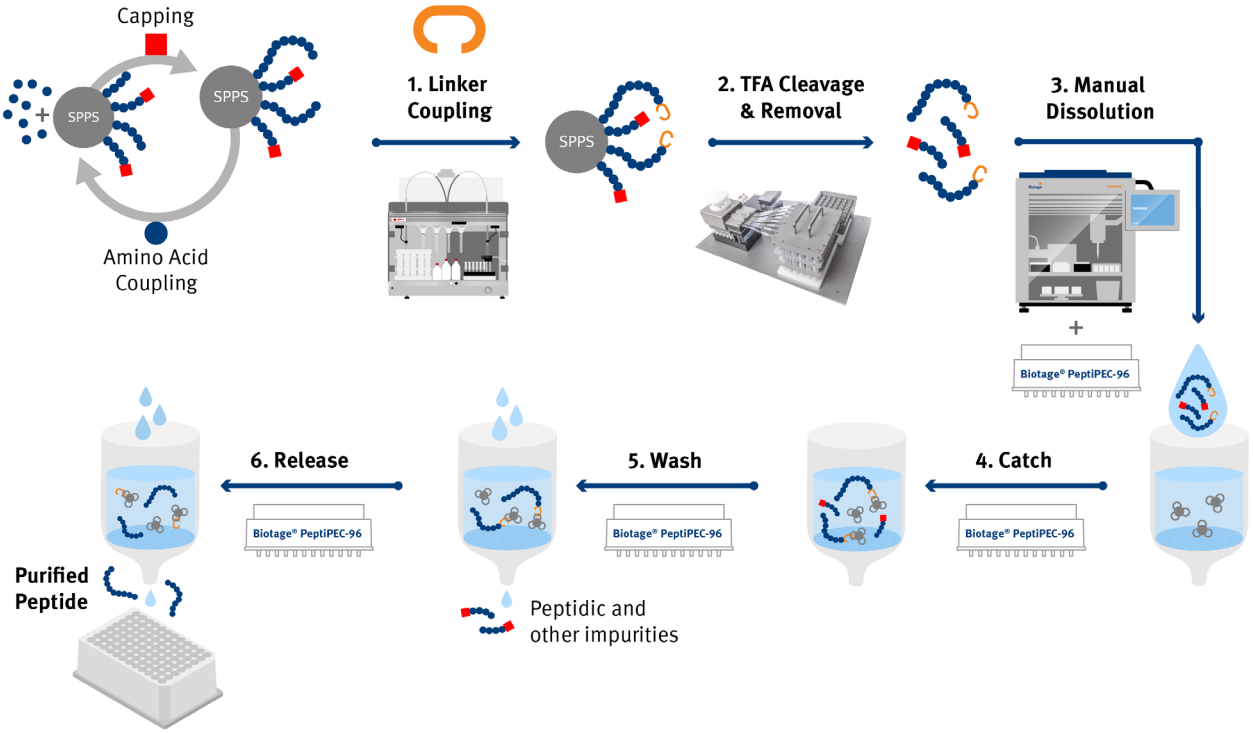
A traditional SPE workflow uses a functionalized sorbent that leverages physical or chemical adsorption interactions to separate sample components. As the sample passes through the sorbent material, desired sample components are retained by the media and subsequently eluted using a compatible solution.

Reversed-phase chromatography with C18-based media is often used in peptide purification, making it a logical choice for peptide SPE applications too. Biotage® PeptiRen-96 plates contain C18 media optimized for use with synthetic peptides, enabling intuitive and reproducible SPE purification.

1. **Synthesis:** Synthesize peptides using Fmoc solid-phase peptide synthesis (SPPS). This can be performed on Biotage's Syro parallel peptide synthesizer.
2. **Cleavage:** Release the peptide from the SPPS resin and utilize Biotage's Syro accessories to facilitate filtration into a 96-well plate. Recover crude peptides as a solid using desired strategy.
3. **Manual Dissolution:** Solubilize the peptides.

   Remaining steps are performed on Biotage® Extrahera™ workstation.
4. **Condition:** Wet (condition) the media to extend C18 chains and prepare for use.
5. **Equilibrate:** Convert the mobile phase conditions around the media to ensure that the peptides are properly retained.
6. **Load:** Add peptides to the Biotage® PeptiRen-96 C18 plate where they are retained by the media.
7. **Wash:** Selectively elute sample impurities from the media while retaining the desired peptides.
8. **Elute:** Selectively release peptides from the media and collect them for further analysis.

How it works: Peptide Purification Process with Biotage® PeptiPEC and Biotage® Extrahera



PurePep® EasyClean (PEC™) for Peptide Purification

Biotage® PeptiPEC-96 high-throughput kit is a catch-and-release technology using a novel, reductively cleavable linker molecule (PEC-Linker RC+) and activated filter materials (aldehyde modified agarose beads). The solution is based on the PurePep® EasyClean (PEC™) technology from Gyros Protein Technologies, and it enables the purification of various peptides, including short, long, hydrophilic, hydrophobic and/or aggregating peptides simultaneously, in a one-method fits all protocol.

1. **Coupling:** Synthesize peptides using Fmoc solid-phase peptide synthesis (SPPS) including a capping step after each amino acid coupling. Attach the PEC-Linker RC+ to the *N*-terminus of the completed peptide chain. This can be performed using Biotage's Syro parallel peptide synthesizer.
2. **Cleavage:** Release the peptide from the SPPS resin and utilize the Syro accessories to facilitate filtration into a 96-well plate. Recover crude peptides as a solid using a desired strategy.
3. **Manual Dissolution:** Dissolve the peptides.

   Remaining steps are performed on Biotage® Extrahera™ workstation.
4. **Catch:** Catch the PEC-Linker tagged target peptides by covalent capture onto the activated filter material.
5. **Wash:** Wash out unbound impurities, such as truncated and deletion sequences and other synthetic impurities.
6. **Release:** Reduce the PEC-Linker under neutral conditions, then apply acid treatment for traceless and safe release of the desired peptides.

Less programming, more processing: Biotage® Extrahera™ Workstation

Biotage® Extrahera™ is an automated sample preparation workstation widely used in forensic, clinical, and doping laboratories worldwide. Renowned for its user-friendly software and flexible column and plate formats for different analytical applications, this workstation is powered by positive pressure, and equipped with optimized protocols. Biotage® Extrahera™ now enables the efficient processing of 96-well plates for peptide library purification.

The workstation features two pipette tip rack positions for reagents/solvents and samples, employing industry-standard automation tips and intelligent tip reuse options. Its compact design optimizes laboratory space, featuring an enclosed processing area and integral exhaust fan for enhanced safety. The clean and intuitive user interface simplifies system setup, minimizing programming time and enabling more efficient processing.

Biotage® Extrahera™ - Peptide

Ordering Information

Part Number	Description
401-0500-PX01	Biotage® PeptiRen-96 C18 100 Å 500 mg Plates
PEC-096-010	Biotage® PeptiPEC-96 High-Throughput Kit
419123SP	Biotage® Extrahera™ - Peptide
419124SP	Configuration Kit for 96 Positions – Peptide

What's in the box?

Biotage® PeptiRen-96

- 96 well plate containing 500 mg of C18 100 Å media in each well

Biotage® PeptiPEC

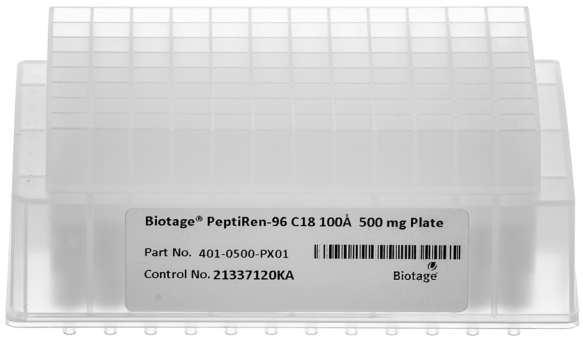
- PEC filter plate (96-well) with activated filter material
- PEC-Linker RC+
- Buffer salt: Mixture of citric acid & sodium carbonate
- Blocking agent: L-Cysteine
- Reducing agent: Dithiothreitol (DTT)
- TFA collection plate (96-well)
- Peptide collection plate (96-well)

Biotage® Extrahera™ - Peptide

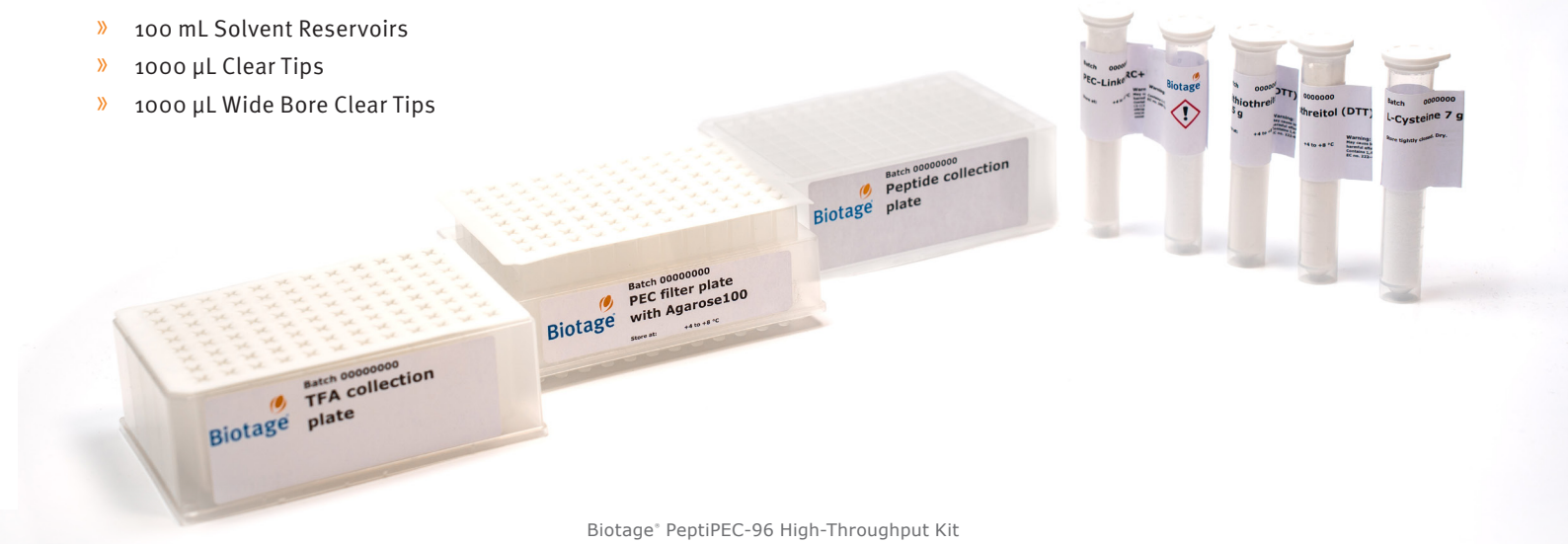
- Biotage® Extrahera™
- Vacuum Pump ME1C
- Accessory Kit, Vacuum pump ME1C

Configuration Kit for 96 Positions - Peptide

- Configuration Kit - 96 Positions Dual Flow
- Solvent Safety Kit - GL45 Caps, Filters, & 1 L Glass Bottle
- 100 mL Solvent Rack
- 100 mL Solvent Reservoirs
- 1000 µL Clear Tips
- 1000 µL Wide Bore Clear Tips



Biotage® PeptiRen-96



Biotage® PeptiPEC-96 High-Throughput Kit

Your Complete Partner for Effective Chemistry

Biotage is a worldwide supplier of instruments and accessories designed to facilitate the work of scientists in life sciences. With our deep knowledge of the industry, academic contacts and in-house R&D teams, we can deliver the best solutions to your challenges. We take great pride in our flexibility and ability to meet our customer's individual needs. With strong foundations in analytical, organic, process, and biomolecule chemistry, we can offer the widest range of solutions available on the market.

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