

Hydrophobic Interaction Chromatography (HIC) Buffer Set

Supplied as powder in two bottles. Dissolve in HPLC-grade deionized water prior to use.

Product Numbers:

CM02025-1000ML

Buffer A: CM02025.1-1000ML

Buffer B: CM02025.2-1000ML

Please consult reconstitution instructions in this document.

Product Description

CellMosaic's HIC HPLC Analysis Buffer Set is designed for the convenient separation and analysis of bioconjugates labeled with highly hydrophobic small molecules based on differences in hydrophobicity. Hydrophobic interaction chromatography (HIC) is a useful technique for characterizing bioconjugates such as antibody-drug conjugates (ADCs) as well as protein- or peptide-drug conjugates. The interaction between the bioconjugates and the column media is influenced by the salt concentration or organic solvent composition of the running buffer.

To support bioconjugate analysis, CellMosaic has developed this high-quality HIC buffer set for use following preparation of bioconjugates with CellMosaic's Personalized Conjugation Kit™ (PerKit™).

A high salt concentration promotes stronger interaction between the bioconjugates and the column media. In a typical HIC method, samples are eluted using a decreasing salt gradient. Under these conditions, bioconjugates with the lowest degree of labeling and therefore lower hydrophobicity elute first, while more highly labeled, more hydrophobic bioconjugates elute later. If the conjugated molecule is hydrophilic, such as CellMosaic's proprietary AqT® molecules, the elution trend is reversed. In this case, bioconjugates with a higher degree of labeling become more hydrophilic and exhibit weaker interaction with the HIC column. As a result, highly labeled species elute earlier, while less labeled, relatively more hydrophobic species elute later under a decreasing salt gradient.

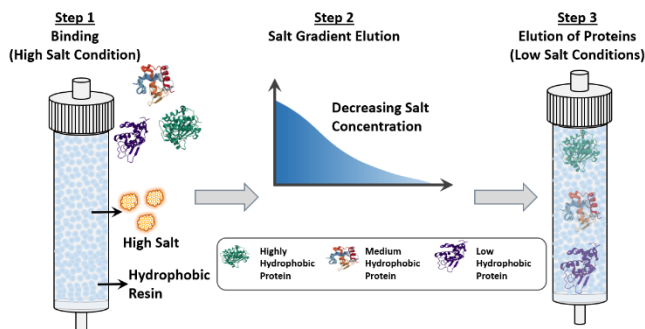
The HIC buffer set **CM02025-1000ML** includes two buffers and instructions for use:

Name	Part #	Volume upon dissolution	Description
HIC Buffer A	CM02025.1-1000ML	1 Liter	0.1M Sodium phosphate buffer, 1.8 M Ammonium sulfate, pH 7.0 ± 0.1
HIC Buffer B	CM02025.2-1000ML	1 Liter	0.1M Sodium phosphate buffer, pH 7.0 ± 0.1

Key Features

- Experimentally selected ultra-pure components ensure minimal UV background absorbance.
- Compatible with all standard HIC columns for biopolymer analysis.
- Each buffer set provides sufficient volume for 120 analyses, depending on usage conditions.
- All buffer bottles and components are sterile.

Hydrophobic Interaction Chromatography (HIC) Principle



Reconstitution and Usage



1. Add **HPLC-grade deionized water** (freshly prepared ultrapure (≥ 18 M Ω ·cm) water suitable for HPLC analysis) to the bottles, up to the 1000 mL mark. For the final 10–20 mL, use a squeeze bottle or pipette to **carefully adjust the volume** to the mark, **reading at eye level**. Cap and mix until fully dissolved. Allow the solution to equilibrate to ambient temperature before use.
2. Prepare your HPLC system. Any standard HPLC or UPLC system is suitable. Flush the system thoroughly with deionized water prior to use.
3. Transfer Buffer A and Buffer B to appropriate HPLC solvent bottles, or use the supplied containers directly if compatible with your system. Supplied bottles are designed to fit standard GL45 caps commonly used in HPLC systems.
4. Prime and flush the HPLC system with the buffers to ensure proper equilibration.
5. Install your HIC column. This buffer set is compatible with all standard HIC columns. Adjust method parameters as needed based on column dimensions (length and diameter) and column volume. *Commonly used columns include Waters Protein-Pak™ Hi Res HIC and Tosoh Ether-5PW.*
6. Set up the HPLC method (see example method below). For improved separation of conjugates, a shallower linear gradient may be used.
7. Run blank injections until a stable, smooth baseline is achieved (typically 2–3 injections).
8. Inject and run your samples.
9. After completing the analysis, replace Buffer A and Buffer B with deionized water. Wash the column with at least 5 column volumes of deionized water. Remove the column and continue flushing the system with deionized water until all residual salt is eliminated.

Example HPLC method:

Equipment: Agilent 1100 equipment.

Column: Waters Protein-Pak™ Hi Res HIC Column, 2.5 μ m, 4.6 mm X 35 mm (Part#: 186007582).

Gradient: Pre-equilibrium of the column for 4 minutes with Buffer A before starting a sample run. The elution method consists of a linear gradient from 100% Buffer A to 100% Buffer B in 10 minutes and then held at 100% B for additional 4 minutes. Flow rate: 0.8 mL/min.

Example HIC profiles:

Figure 1: HIC HPLC analysis of MMAE-ADC with VC-PAB linker (conjugates made and purified using a kit from CellMosaic, CM11409) using the example method.

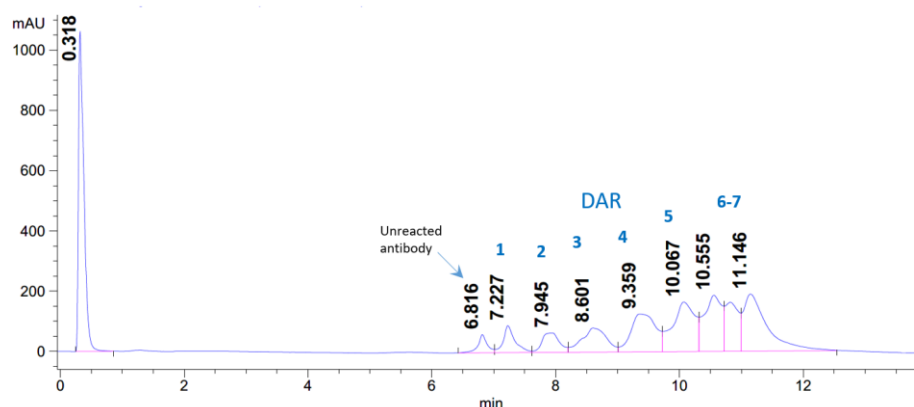
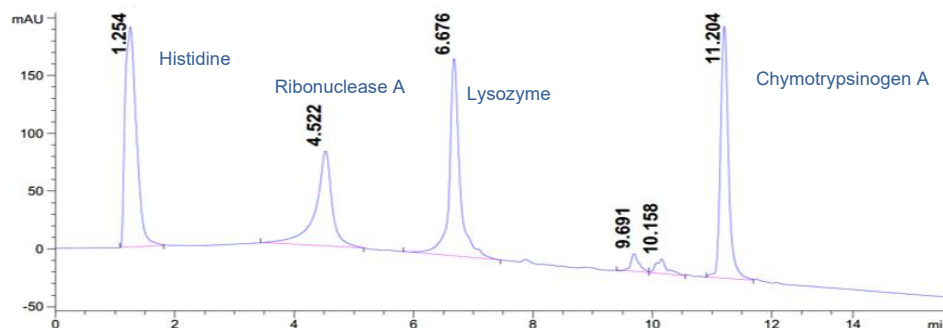


Figure 2: Separation of HIC protein standard (CM92007) using a Waters Protein-Pak™ Hi Res HIC column (4.6 mm x 100 mm, 2.5 µm, Part No. 186007583). HPLC condition: CellMosaic's HIC Buffer set - CM02025 with 15 min gradient at a flow rate of 0.6 mL/min.



Applications of HIC

- **Antibody-Drug Conjugates (ADCs) & Bioconjugates**
DAR determination, conjugation heterogeneity, species separation.
- **Protein Purification (Preparative HIC)**
Capture and polishing in downstream processing.
- **Protein Variant and Isoform Separation**
Separation of isoforms and subtle structural variants.
- **Protein Folding and Conformation Studies**
Monitoring folding/unfolding and conformational changes.
- **Aggregate Analysis**
Detection of hydrophobic aggregates.
- **Viral Vector and Vaccine Purification**
Purification of viral particles and vaccine proteins.
- **Peptide and Protein-Drug Conjugates**
Analysis of peptide-drug and protein-small molecule conjugates.
- **Biosimilar and Biologic Comparability**
Comparing originator vs biosimilar products.

- **Quality Control in Biopharmaceutical Manufacturing**

Process monitoring and batch consistency.

- **Enzyme Purification and Characterization**

Isolation of enzymes and isoenzymes.

Storage and Solution Stability

- **Undissolved HIC Buffer A and Buffer B** are stable at ambient temperature for up to 5 years.
- **Reconstituted HIC Buffer A** should be stored at room temperature and is stable for up to 1 year. Do not store at 2–8 °C to avoid salt crystal formation.
- **Reconstituted HIC Buffer B** can be stored at ambient temperature for up to 1 week. For longer-term storage, store at 2–8 °C to prevent microbial or algae growth.

Key References

Antibody-Drug Conjugates (ADCs) & Bioconjugates:

Chen, X. et al., *mAbs*, 2016, 8(7): 1210–1223.

Highlight: Demonstrates that HIC enables **high-resolution separation of ADC species by DAR**, making it a **primary analytical method for ADC characterization**.

Protein Purification (Preparative HIC):

Queiroz, J.A. et al., *Journal of Biotechnology*, 2001, 87(2): 143–159.

Highlight: Shows HIC as a **mild, non-denaturing purification technique** that preserves protein structure and achieves high recovery.

Protein Variant and Isoform Separation:

Fekete, S. et al., *Journal of Pharmaceutical and Biomedical Analysis*, 2014, 101: 161–173.

Highlight: Illustrates that HIC can resolve **monoclonal antibody variants based on subtle hydrophobic differences**, complementing other analytical methods.

Protein Folding and Conformation Studies:

Fausnaugh, J.L., Kennedy, L.A., Regnier, F.E., *Journal of Chromatography A*, 1984, 317: 141–155.

Highlight: Shows that **unfolded or partially folded proteins exhibit stronger retention** due to exposed hydrophobic regions.

Aggregate Analysis:

Arakawa, T. et al., *Protein Science*, 2006, 15(6): 1487–1495.

Highlight: Highlights HIC's ability to detect **hydrophobic aggregates not easily resolved by size-exclusion chromatography (SEC)**.

Viral Vector and Vaccine Purification:

Kalbfuss, B. et al., *Journal of Chromatography B*, 2007, 849(1–2): 94–104.

Highlight: Demonstrates HIC as an effective method for **purifying viral particles while maintaining biological activity**.

Peptide and Protein-Drug Conjugates:

Wakankar, A.A. et al., *Bioconjugate Chemistry*, 2011, 22(3): 449–456.

Highlight: Shows HIC can **separate conjugates based on hydrophobic payload loading**, similar to ADC DAR analysis.

Biosimilar and Biologic Comparability:

Beck, A. et al., *Trends in Analytical Chemistry*, 2013, 48: 81–95.

Highlight: Identifies HIC as a tool to detect **subtle hydrophobic differences between originator biologics and biosimilars**.

Quality Control in Biopharmaceutical Manufacturing:

Shukla, A.A., Thommes, J., *Trends in Biotechnology*, 2010, 28(5): 253–261.

Highlight: Highlights HIC as a **robust and scalable method for monitoring product consistency and impurities** in manufacturing.

Enzyme Purification and Characterization:

Geng, X., Wang, F., *Journal of Chromatography A*, 1993, 640(1–2): 345–351.

Highlight: Demonstrates effective **enzyme separation based on surface hydrophobicity while maintaining catalytic activity**.

Important Notes

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