CellMosaic, Inc.
10A Roessler Road
Woburn, MA 01801, USA
Phone: 781-463-0002; Fax: 781-998-4694; E-mail: info@cellmosaic.com

Hydrophobic Interaction Chromatography (HIC) Buffer Set

Product Number: **CM02025** (Buffer A: CM02025.1 and Buffer B: CM02025.2)

Product Description

HIC HPLC analysis is a useful tool to separate bioconjugates labeled with very hydrophobic small molecules based on their hydrophobicity. The hydrophobic interaction of the bioconjugates and the column media is affected by the presence of salts or organic solvent in the running buffer.

CellMosaic has designed this high quality HIC buffer set for your convenient analysis of bioconjugates, such as antibody-drug conjugate (ADC) and protein or peptide-drug conjugate following the preparation of the bioconjugates using CellMosaic's Personalized Conjugation Kit™ (PerKit™).

This HIC buffer set comes with two buffers and instruction for usage. **Buffer A** has high salt and **Buffer B** contains no salt. A high salt concentration aids the interaction of the bioconjugates with the column media. Usually, a decreasing salt gradient is used to elute samples from the column. As such, the lowest degree of labeled bioconjugate (low hydrophobicity) is eluted first and the most labeled bioconjugates elute last.

Quality and Convenience

- Experimentally selected ultra-pure components for minimum UV background absorbance.
- Sterile filtrated & bottled to prevent any analyte degradation during the run
- Can be used with any standard HIC column for biopolymer analysis
- Each buffer set is **sufficient for 30, 60, or 120 analysis**

Application

- Analyze antibody-drug conjugates
- Analyze protein and peptide-drug conjugates
- Analyze other biopolymers labeled with very hydrophobic small molecules

Reference: Hydrophobic interaction chromatography for the characterization of monoclonal antibodies and related products. Szabolcs Feketea; Jean-Luc Veuthey; Alain Beck; Davy Guillarme. *Journal of Pharmaceutical and Biomedical Analysis* 130 (2016) 3–18.

Key Components

Two buffer bottles per package. Number of Injections per buffer set: CM02025-250 mL (30 injections); CM02025-500 mL (60 injections); CM-02025-1L (120 injections).

Name	Part #	Quantity	Description
Buffer A (Red label)	CM02025.1	250 mL, 500 mL, or 1L	0.1M Sodium phosphate buffer, 1.8 M Ammonium sulfate, pH 6.4
Buffer B (Blue label)	CM02025.2	200 mL, 400 mL, or 800 mL	0.1M Sodium phosphate buffer, pH 7.0

Storage/Stability

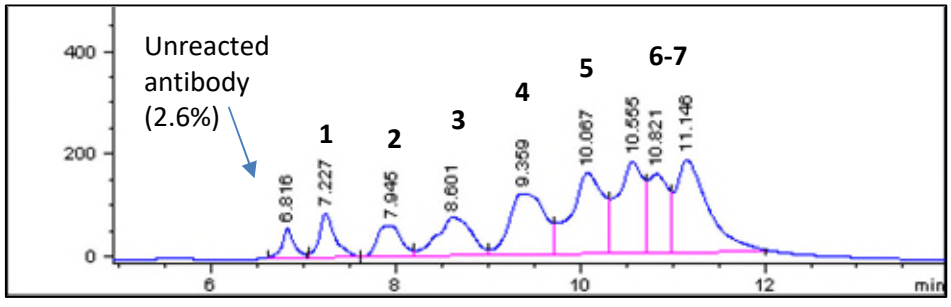
- CellMosaic's HIC **Buffer A** and **Buffer B** are stable at ambient temperature. For long-term storage, please store **Buffer B** at 2-8°C.

Procedure/Example Method

1. Take buffer bottles out and warm to ambient temperature (if stored at 2-8°C).
2. Locate your HPLC equipment. Any standard HPLC equipment including UPLC will be fine. Flush the system with deionized water.
3. Transfer the buffers to HPLC bottles or use as is.
4. Flush the system with buffers.
5. Connect your HIC column. Any HIC column will work with this buffer set. Depending on the column diameter and length, you may need to adjust the example HPLC method to reflect the column volume. (Commonly used column: Waters Protein Pak Hi Res HIC or Tosoh Ether-5PW columns).
6. Set up the method (an example method is shown below). For better separation of the conjugates, you can adjust the linear gradient to be more shallow.
7. Run blanks until the baseline is smooth (usually 2 or 3 blanks).
8. Run your samples.
9. After finishing the run, replace buffer A and B with deionized water. Wash your column with 5 column volumes of deionized water, and then remove the column and continue flushing your system with deionized water until all of the salt is removed.

Example HPLC method:
Equipment: Agilent 1100 equipment
Column: Waters ProteinPak Hi Res HIC 2.5 µm, 4.6x35 mm (Part#: 085Y15020VE07)
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 another 4 minutes.
Flow rate: 0.8 mL/min

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



Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

The information provided in this document and the methods included in this package are for information purposes only. CellMosaic provides no warranty of performance or suitability for the purpose described herein.

Sample data are provided for illustrative and example purposes only and represent a small dataset used to verify kit performance in the CellMosaic laboratory. Information about the chemicals and reagents used in the kit are provided as necessary.

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