

## **Product Information | Certification of Analysis**

## **| Product Information**

mAB pH Gradient Buffer | LC Buffer

Part No.	Name	Size
HLS PH01LCA	LC pH Gradient Buffer A	6.1 g
HLS PH01LCB	LC pH Gradient Buffer B	6.65 g

CAS of Buffer A: 1266615-59-1-1132-61-2 CAS of Buffer B: 2915-38–6-73463-39-5 Lot No.

**Description:** pH gradient buffer is an innovative elution buffer (or mobile phase) that changes pH linearly for separation and purification of mAb antibodies, glycoproteins and PTM modified proteins by ion exchange (IEX) chromatography. In pH gradient buffer-based IEX chromatography, the pH of the initial buffer is kept at a constant level to ensure that the charged proteins bind to oppositely charged ions of the stationary phase. Then, the protein is eluted by linearly changing the pH of the buffer so that the protein has a zero net charge and can elute from the column.

Physical Appearance: Lyophilized powder

Reagent Composition: Each bottle contains 2.5 mg BT Surfactant, 850 mg NaCl, and 6 g sulfonate.

**Resuspension:** Dissolve reagents in 500 mL deionized, double-distilled water.

**pH Adjustment:** At room temperature, use pH meter to ensure the pH error is less than 0.2, and use 2 M NaOH or hydrochloric acid to adjust the pH.

Elution Buffer (mAb): pH Buffer A, pH 5.5

Elution Buffer B (maB): pH Buffer B, pH 10.2

Storage Conditions: In 4 °C refrigerator as lyophilized powder.

Shelf Life: 24 months at 4 °C as powder; 7 days at 4 °C as solution.

Linear pH Range: Linear correlation coefficient > 0.99 at pH 5.5 - 10.2 in columns of IEX chromatography.

Reference Procedure of mAb subunit and intact protein: Column: SCX, 10  $\mu$ m, 2.1 \* 250 mm or 2.1 \* 50 mm

Eluent A: 1X mAb pH buffer A (pH 5.5) Gradient: 0 -1 min, 0% B; 1 - 31 min, 0 - 100% B; Temperature: 30 °C UV detection: 280 nm Inject volume: 4 µL Flow rate: 0.3 mL/min Eluent B: 1X maB pH Buffer B (pH 10.2) 31 - 34 min, 100% B; 34 -40 min, 0%B FLR detection: Ex: 280 nm, Em: 360 nm Load: 0.2 - 10 µg

## **I Quality Control**

Purity: In ion mobility buffer, the BT Surfactant is mass spectrometry reagents, and other components are pure chromatography reagents with < 0.01 absorbance at 280 nm, analyzed by liquid chromatography.</li>
pH Accuracy: pH 5.5 for mAb pH Buffer A and pH 10.2 for maB pH Buffer B with error less than 0.2.
pH Elution Linearity: The linear correlation coefficient of 227 nm absorbance > 0.999 with zero volume injection and SCX ion exchange chromatography system in 30 min run time at 30 °C column temperature.
pH Elution Degree of Seperation: Linear elution degree of separation > 1 with 5 pKa protein mAb: IGg A1, Ribonulease A, Ribonuclease B, and Cytochrome C.





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