New High-Capacity Cellufine™ Phosphate HC Resin Enables Efficient Purification of T7 RNA Polymerase and Other Large Biomolecules

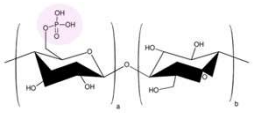
Ayana Uehara[1], Junya Toba[1], Toru Kurabayashi[1], Masami Todokoro[2], Malcolm G. Pluskal [3], Yoshihiro Matsumoto[1]

1 JNC Corporation, Yokohama R&D Center, 2 JNC Corporation, Sales and Marketing Department Life Chemicals Division and 3 JNC America, Cellufine Application Lab, Leominster, MA

Introduction

Cellufine™ resins are spherical, porous cellulose particles that are surface-modified with various chromatography chemistries. They are ideal for purifying biologics such as antibodies, vaccines, and therapeutic proteins. T7 RNA polymerase (T7 RNAP) is an enzyme that is important for production of mRNA transcripts from DNA templates For example, in large-scale production of mRNA vaccine against COVID-19. In this study, we introduce a new Cellufine™ Phosphate HC resin that has much higher binding capacity for T7 RNAP. This resin is very effective for purifying not only T7 RNAP but also other large nucleic binding proteins. We also confirmed that the new resin demonstrated more efficient purification of T7 RNAP from E. coli cell lysates than existing products.

JNC has developed new high-capacity Cellufine™ Phosphate HC.



For Capturing the enzyme

- ✓ No need His-tag
- ✓ Effective for the purification of nucleic acid-binding proteins
- ✓ Low non-specific adsorption

Challenges : The capacity for large enzymes

Purification Outline

Freeze/Thaw to create Cell-free Lysate

↓

Ammonium Sulfate precipitation

↓

Cellufine™ MAX DEAE
(DNA and contaminated protein Reduction)

↓

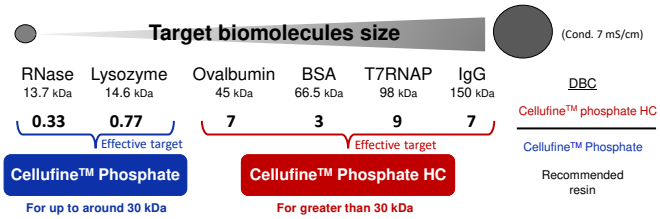
Cellufine™ Phosphate
(RNAP capture and Elution)

↓

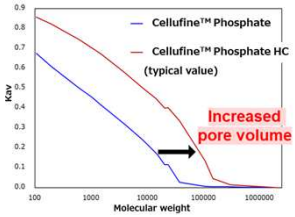
Cellufine™ ET Clean L
(Endotoxin Reduction)

Features of Phosphate and new high-capacity Phosphate HC Resin

◆ Concept



◆ Key information

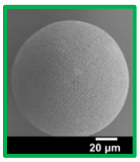


- ➢ Particle size, ligand and ligand density similar for both Phosphate resins.
- ➢ New high capacity Cellufine phosphate HC has increased pore volume

✓ **Cellufine™ Phosphate** is effective for the adsorption of **small molecules**, and the new high-capacity **Cellufine™ Phosphate HC** is effective for **large molecules** > 30 kDa.

◆ Base resin

standard beads

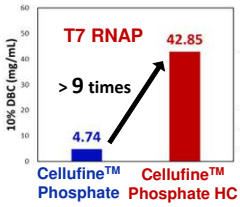


specific surface area(BET method)

	m²/mL
Cellufine™ phosphate	17.98
Cellufine™ phosphate HC	15.97

✓**The original base resins are the same.**
✓**The specific surface area per volume is also almost the same.**

◆ Comparison of Dynamic Binding Capacity (DBC)



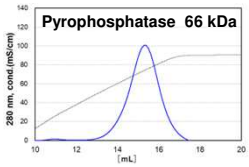
5%DBC

Cond.: 7 mS/cm
Retentiont :2 min
MWt: 98 kDa

Figure 1. Results of DBC for Nucleic Acid-Binding proteins (enzyme)

✓**The DBC of the new Cellufine™ Phosphate HC is 9 times higher than the Cellufine™ Phosphate for the large enzyme T7 RNAP.**

◆ Affinity for Phosphate



Elution at **74.5** mS/cm

Pyrophosphatase:
Commercially available
Buffer: PB pH7
Column volume:1.06mL

T7 RNAP purification

◆ Step1: Cellufine™ MAX DEAE

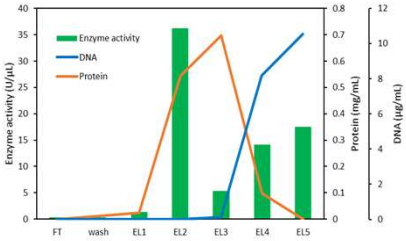


Figure 2. Cellufine™ MAX DEAE fractionation of T7 RNAP crude lysate after Ammonium sulfate precipitation

Equilibration buffer : 10 mM Tris-HCl pH 7.5, 50 mM NaCl, 0.1 mM EDTA, 0.5 mM DTT, 10% (v/v) glycerol + Protease inhibitors
Elution buffer : 0.5 M NaCl in the equilibration buffer
Column Volume : 1.06 mL
Flow rate : 0.5 mL/min (Retention time 2 min)

◆ Step2: Cellufine™ Phosphate

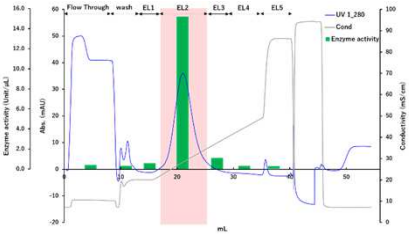
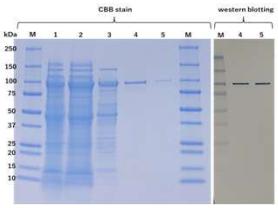


Figure 3. Elution of T7 RNAP from Cellufine™ Phosphate with a linear salt gradient

Equilibration buffer : 10 mM potassium phosphate pH 7.5, 50 mM NaCl, 0.1 mM EDTA, 0.1 mM DTT + Protease inhibitors
Elution buffer : 0.5 M NaCl in the equilibration buffer
Column Vol : 1.06 mL
Flow rate : 0.5 mL/min (RT 2 min)

Table 1.Residual impurities in prototype HC and current phosphate resins

	Elution Enzyme activity(U)	Protein ng/Unit	DNA pg/Unit
Load	-	5.27	1.14
Cellufine™ phosphate	626816	1.53	0.58
Cellufine™ phosphate HC	603621	1.69	0.49



M: marker
1: Lysate
2: Ammonium sulfate precipitate
3: Cellufine™ MAX DEAE elution
4: Cellufine™ Phosphate elution
5: Positive control

Figure 4. Confirmation of T7 RNAP purity by SDS-PAGE and Western blotting

- ✓ Cellufine™ Phosphate HC can **retain T7 RNAP from post Cellufine™ MAX DEAE elution fraction**.
- ✓ SDS-PAGE and Western blotting analysis of Cellufine™ Phosphate elution showed **single band of T7 RNAP at 98 kDa (Figure4).**
- ✓ Cellufine™ phosphate HC elution fraction showed a **high specific enzyme activity and recovery**.
- ✓ Both Cellufine™ Phosphate and New high-capacity Phosphate HC can achieve **high clearance of impurities in the final T7 RNAP enzyme.**

Conclusion

In this poster we were able to purify T7 RNAP enzyme using new high-capacity Cellufine™ Phosphate HC resin.

- New Cellufine™ Phosphate HC resin provides **significantly enhanced binding capacity for large nucleic acid binding proteins such as T7 RNAP and Pyrophosphatase.**
- New Cellufine™ Phosphate HC can **reduce impurities to a level comparable to existing products**, enabling efficient purification of T7 RNAP.
- Efficient purification of T7 RNAP from E. coli was achieved by **combining Cellufine™ MAX DEAE and Cellufine™ ET Clean L with the new resin**.

For more information, please visit our website.

Presented at BPI Boston 2025

Samples are available!