The use of Capto Core 700 and Capto Q ImpRes in the purification of human papilloma virus like particles

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The use of Capto™ Core 700 and Capto Q ImpRes in the purification of human papilloma virus like particles

This application note describes the results of a project executed by the GE Healthcare BioProcess™ Services team to display proof of principle of a purification procedure for human papillomavirus (HPV) L1 protein, the antigenic component of HPV vaccines. Modified L1 protein was expressed in insect Sf9 cells using baculovirus vector, and allowed to spontaneously assemble into virus like particles (VLPs). A modern, scalable approach, based on two chromatographic steps including novel chromatography media (resins), was used for the purification of L1 protein. In the first step, VLPs are purified in flow-through mode using Capto Core 700, a layered-bead size exclusion medium. In the second step, Capto Q ImpRes medium allows polishing of the L1 protein with high purity and resolution.

Introduction

HPV is a common sexually transmitted infection in adults. More than 111 genotypes of HPV have been described, among which about 30 have been associated with anogenital cancers. Several genotypes are also classified as low risk and associated with benign lesions and genital warts.
To prevent cervical cancer and to reduce the number of treatments for cervical cancer precursors, the World Health Organization (WHO) recommends vaccination of young women against HPV (1). Current vaccines protect against up to four virus genotypes. However, vaccines protecting against more genotypes would prevent more cancer forms and be less susceptible to regional variations of the virus. In this work, HPV 58, a subtype commonly occurring in Asia (2), was used as vaccine immunogene. As papillomaviruses are not easily grown in cell cultures, the vaccine constructs are VLP-based, most commonly with recombinant L1 protein

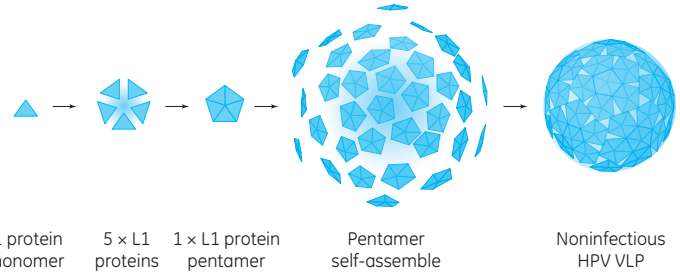

Fig 1. Schematic picture of formation of L1 proteins into HPV VLP.

as the structural component. L1 proteins spontaneously self-assemble to hollow VLPs that resemble authentic HPV virons. The VLP vaccine lacks the viral nucleic acid and, hence, cannot induce infection. Nevertheless, the vaccine triggers an immunogenic response that protects against HPV infection. Here, the structural L1 protein is expressed in insect Sf9 cells using a baculovirus vector. Five L1 protein units form pentamers, which in turn self-assemble into VLPs (Fig 1). The diameter of the VLP is about 55 nm.

To reach adequate purity, previously established purification processes combines anion exchange, hydrophobic interaction, and hydroxyapatite chromatography. Here, we describe a simple procedure to separate the VLPs from host cell-derived impurities to high purity using two orthogonal chromatographic steps: layered-bead size exclusion and anion exchange chromatography. The tentative purity target was set to > 95% as determined by densitometry analysis of electrophoresis gels. Protein purity was calculated as the L1 protein-to-total protein ratio and expressed as a percentage.

Capto Core 700 represents a new generation of chromatography media and is designed and optimized for purification of viruses and other large biomolecules. The

bead has octylamine ligands coupled to the core that is surrounded by an inert outer layer. Large target molecules (> M_r 700 000) pass outside the bead, while smaller molecules can bind to the inner core (Fig 2).

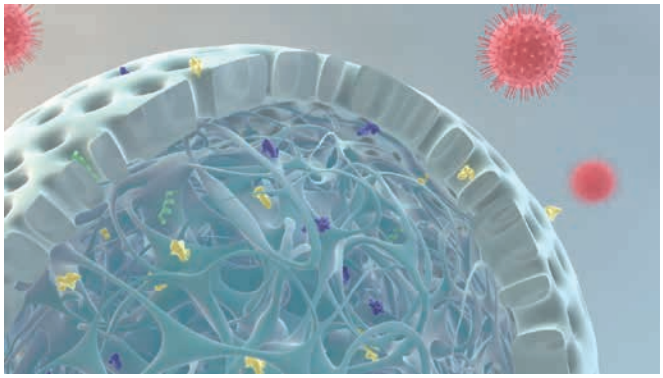

Fig 2. Schematic representation of Capto Core 700 showing a bead with the inactive, porous shell and the ligand-containing core. Proteins and impurities (colored green, yellow, and purple) penetrate the core, while target viruses (red) and larger biomolecules (> M_r 700 000) are excluded from the medium and pass in the flowthrough.

In this work, Sf9 cells, used for production of the L1 protein, were lysed under mild stirring in buffer, whereupon the supernatant was centrifuged and filtered to remove cell debris. The filtrate was run in flow-through mode on Capto Core 700 medium for removal of impurities. After passing the Capto Core 700 column, the VLPs were disassembled into L1 protein monomers by disruption of disulfide bonds between the L1 proteins using dithiothreitol (DTT). In the second chromatographic step, the L1 protein was purified in bind-and-elute mode on Capto Q ImpRes medium. Capto Q ImpRes is a strong anion exchanger for high-throughput intermediate purification and polishing of a wide range of biomolecules. The small particle size of Capto Q ImpRes enables high resolution, which is combined with high-flow capabilities derived from the mechanical stability of the Capto base matrix. After this purification procedure, the L1 protein can be allowed to spontaneously reassemble into VLPs again by removal of the DTT (Fig 3).

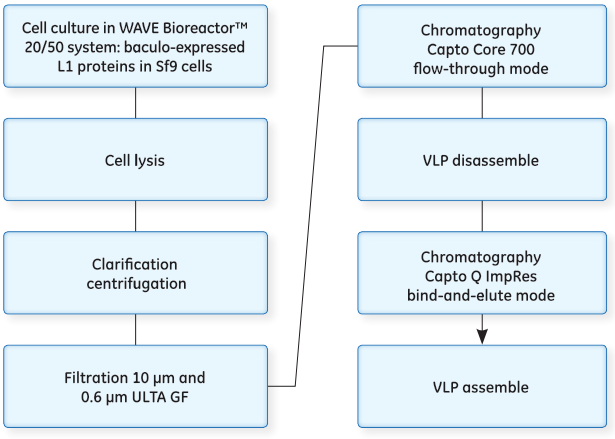

Fig 3. Schematic picture of the purification procedure.

Materials and methods

Protein expression

Sf9 cells, infected with baculovirus containing the L1 protein gene, were cultured in a 20 L Cellbag™ bioreactor using a WAVE Bioreactor 20/50 system at 15 to 17 rpm rocking speed and 7° angle. The culture process was monitored with a WAVEPOD™ II controller for 15 days. InsectExpress Prime including L-glutamine and SFX-Insect cell culture media were used. Cells were washed with phosphate-buffered saline (PBS) to remove the major part of the cell culture media, and pelleted by centrifugation. The cell pellet was stirred with 20 mM Tris, pH 8.5 for 1 h followed by addition of 300 mM NaCl and continued stirring for another 30 min. The treatment resulted in hypotonic cell lysis.

Clarification

The cell lysate was centrifuged at 10 000 × g for 30 min, whereupon the supernatant was removed and centrifuged for an additional 10 min at the same speed. Finally, the feed was filtered using a combination of a 10 micron and a 0.6 micron ULTA™ Disc GF 47 mm filter membrane.

Chromatography

The self-assembled VLPs, extracted from the cells, were purified in two chromatography steps. First, host cell-derived impurities were removed using Capto Core 700 and the VLPs were collected in the flowthrough. For optimizing protein removal, sample load of the clarified lysate was evaluated in a prestudy. Based on the results from the prestudy, about 8 mg protein/mL medium was loaded for optimized protein removal. For disruption of the VLP structure to enable monomeric L1 proteins, the flowthrough from Capto Core 700 was incubated with 20 mM DTT in Tris-buffer, pH 8.5 for 15 min and filtered using a 0.6 micron ULTA Disc GF 47 mm filter membrane. The L1 protein was finally captured on Capto Q ImpRes and eluted using a stepwise gradient of increasing conductivity. Detailed information on the two chromatographic steps is summarized in Table 1.

Analytical methods

SDS-PAGE was performed using Novex™ NuPAGE™ Bis-Tris (4% to 12%) precast gels (Life Technologies) with reducing agent. The gels were run at a constant voltage of 120 V until the leading marker reached the end. The gels were stained with SimplyBlue™ Safe-Stain solution (Life Technologies). The stained gels were scanned using Image Scanner III and analyzed using ImageQuant™ TL software. Protein bands cut out from the electrophoresis gels were analyzed with mass spectrometry.

Total protein analysis was performed using Modified Lowry Protein Assay Kit (Thermo Scientific) with BSA as standard. Optical density was measured with SpectraMax™ M2 (Molecular Devices).

Table 1. Overview of the two chromatographic steps of the L1 protein purification procedure

	Step 1	Step 2
Column	Capto Core 700, XK 26/10, column length = 10 cm, column volume (CV) = 53 mL (flow-through mode)	Capto Q ImpRes, Tricorn™ 10/150, column length = 15 cm, CV = ~ 12 mL (bind-and-elute mode)
Sample	Clarified insect cell supernatant	Flowthrough from step 1, diluted 3-fold with 20 mM Tris, 20 mM DTT, pH 8.5 and filtered (0.6 µm)
Sample load	~ 1 CV of cell lysate, ~ 8 mg protein/mL medium at 150 cm/h	3 CV of flowthrough, 150 cm/h
Running buffer	20 mM Tris, 300 mM NaCl, pH 8.5, 200 cm/h	20 mM Tris, 100 mM NaCl, 20 mM DTT, pH 8.5, 250 cm/h
Wash	20 mM Tris, 300 mM NaCl, pH 8.5, 150 cm/h	20 mM Tris, 100 mM NaCl, 20 mM DTT, pH 8.5, 150 cm/h
Elution	-	Step gradient to 9.3%, 16.5%, and 100% 20 mM Tris, 1 M NaCl, 20 mM DTT, pH 8.5, 150 cm/h
Cleaning in place (CIP)	1 M NaOH, 30% 2-propanol followed by 2 M NaCl, 60 cm/h	1 M NaOH, 100 cm/h
System	ÄKTA™ avant 150	ÄKTA avant 25
Product	VLP and baculovirus	L1 protein

Results and discussion

DNA and endotoxin removal was not evaluated in this study. Here, the purity goal for the L1 protein was set to > 95%.

Chromatography using Capto Core 700 (flow-through mode)

Analysis of the total protein content confirmed that more than 80% of the protein was removed by Capto Core 700. The SDS-PAGE gel provides a visual image of the removal of proteins. Results from the first Capto Core 700 purification step are displayed in Figure 4.

Chromatography using Capto Q ImpRes (bind-and-elute mode)

Reducing agent (DTT) was added to the flowthrough from the first chromatography step to disassemble the VLPs before loading on the Capto Q ImpRes column. The L1 protein was allowed to bind to the anion exchanger and eluted with increased conductivity (9.3% elution buffer, ~ 184 mM NaCl) from Capto Q ImpRes (P1), followed by two additional elution steps with 16.5% (P2) and 100% (P3) elution buffer. Results from the Capto Q ImpRes chromatography step are shown in Figure 5.

Analysis of the band intensity from the SDS-PAGE gel using ImageQuant software confirmed that the purity of the L1 protein after the polishing step (P1) was high, over 99%. The result is well in line with the study objective.

Column: Capto Core 700, XK 26/10
Sample: Clarified insect cell supernatant
Sample load: ~ 1 CV of cell lysate, ~ 8 mg protein/mL medium at 150 cm/h
Running buffer: 20 mM Tris, 300 mM NaCl, pH 8.5, 200 cm/h
CIP: 1 M NaOH, 30% 2-propanol followed by 2 M NaCl, 60 cm/h
System: ÄKTA avant 150

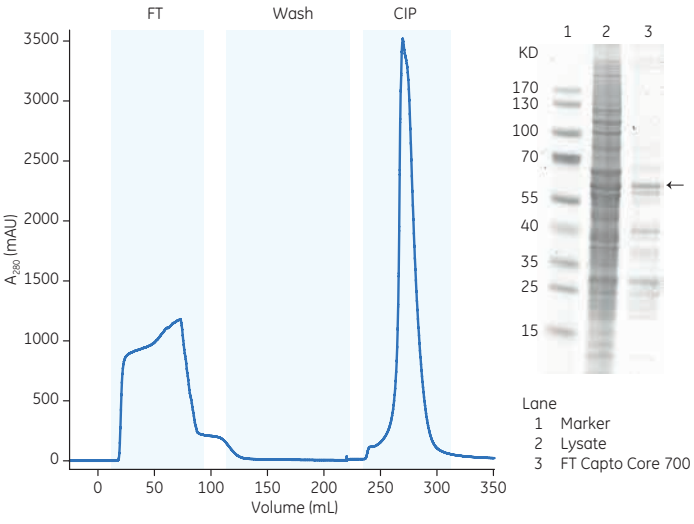

Fig 4. Separation of VLPs from small protein impurities using Capto Core 700 packed in a XK 26/10 column. The VLPs were collected in the flowthrough (FT). SDS-PAGE analysis demonstrates composition of the sample before and after the Capto Core 700 step. Position of the target L1 protein (arrow) was confirmed by mass spectrometry.

Column Capto Q ImpRes, Tricorn 10/150
Sample Flowthrough from Capto Core 700
Sample load: 3 CV of flowthrough, 150 cm/h
Running buffer: 20 mM Tris, 100 mM NaCl, 20 mM DTT, pH 8.5, 250 cm/h
Wash: 20 mM Tris, 100 mM NaCl, 20 mM DTT, pH 8.5, 150 cm/h
Elution: Step gradient to 9.3%, 16.5%, and 100% 20 mM Tris, 1 M NaCl, 20 mM DTT, pH 8.5, 150 cm/h
System ÄKTA avant 25

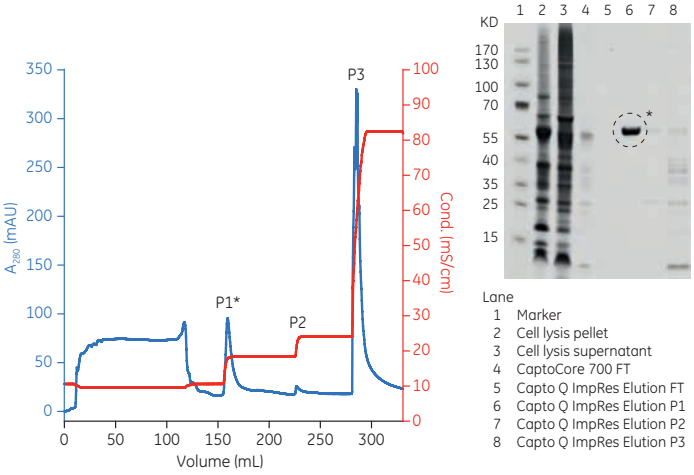

Fig 5. Polishing using Capto Q ImpRes packed in a Tricorn 10/150 column. The disassembled VLPs were loaded on the column under reducing conditions to allow the L1 protein to bind the anion exchanger. The flowthrough and elution peaks (P1, P2, and P3) were collected and analyzed by SDS-PAGE. ImageQuant software was used for densitometry analysis of purity of the L1 protein (*).

The main structural proteins of baculovirus are summarized in an article by Rohrmann (3). These proteins are likely to correspond with the band patterns seen in P3 in Figure 5.

The experiments described in this application note show how HPV L1 protein can be efficiently purified using a combination of Capto Core 700 and Capto Q ImpRes media. From this material, VLPs can be reassembled by removal of the reducing agent DTT. Removal of DTT can be performed, for example, by a buffer exchange step using a Sephadex™ G-25 column or by diafiltration using a hollow fiber filter setup (UFP 10 000NMWC). The same hollow fiber filter can be used to remove excess buffer by ultrafiltration to the desired target concentration.

Conclusion

This application note describes a procedure for the purification of HPV L1 protein by orthogonal separation principles using Capto Core 700 and Capto Q ImpRes. Capto Core 700 offers effective separation by size, where VLPs are allowed to pass in the flowthrough, whereas impurities are captured in the core of the beads. In the second anion exchange step, L1 protein from disassembled VLPs is captured on Capto Q ImpRes medium. Elution of the target protein from Capto Q ImpRes resulted in a highly purified L1 protein.

References

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2. Bao Y.P., Li N., Smith J.S., Qiao Y.L. Human papillomavirus type distribution in women from Asia: a meta-analysis. *Int J Gynecol Cancer* **18**, 71–79 (2008)
3. Rohrmann G.F. Baculovirus structural proteins. *Journal of General Virology*. **73**, 749–761 (1992)

Order information

Product	Quantity	Code number
Capto Core 700	25 mL	17-5481-01
Capto Q ImpRes	25 mL	17-5470-01

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GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

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