LenioBio

Expressing Virus-Like Particles using the ALiCE® Cell-Free Protein Expression System

Introduction

Virus-like particles (VLPs) as vaccines represent the best of two worlds – they trigger robust and lasting immune responses due to their resemblance to viruses while posing fewer safety concerns due to the lack of genetic material and molecular machinery required for viral replication (1). However, despite this uniqueness, longer time-to-approval for new VLPs hinders their wider adoption as a commercially-viable vaccine platform (2).

To circumvent this issue, already-approved VLPs can be used as carriers to present antigens of other viruses (3). Here we describe the production of a high-quality Hepatitis B core (HBc) carrier VLP using the ALiCE cell-free protein expression system. We further show these VLPs to induce an immune response. This establishes ALiCE as a tool for rapid screening and production of VLP-based vaccine (candidates).

ALiCE is a scalable eukaryotic cell-free protein expression system capable of producing even the most complex proteins in under 48 hours. The proprietary cell-free lysate derived from *Nicotiana tabacum* c.v. BY-2 cells contains all of the machinery necessary to implement eukaryotic post-translational modifications, without specific optimizations. Learn more about ALiCE at www.leniobio.com/technology

Materials and Methods

Creation of HBc VLP-expressing constructs

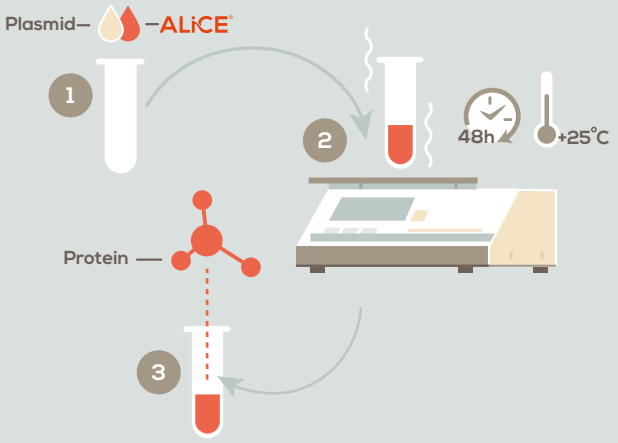
Nicotiana tabacum codon-optimized HBc VLP (adw2 serotype) sequences were cloned into the pALiCE01 vector for cytosolic expression, via Gibson assembly. Three constructs were prepared:

- Untagged full-length HBc VLP sequence (HBc)
- Full-length HBc sequence with N-terminal Strep-II tag (S-HBc)
- Full-length HBc with Strep-II tag on spike sequence (HBc-S)

Expression plasmids were purified from *Escherichia coli* DH5 alpha cultures using the NucleoBond™ Xtra Maxi kit (Prod ref. 740414, Macherey-Nagel™), and their correct assembly was confirmed by sequencing.

Cell-free expression reaction

1 Small-scale BYL CFPS reactions of 50 µl and 100 µl were performed as suggested in the ALiCE user manual. The lysate was thawed on ice and expression plasmids were added to a final concentration of 5 nM. Reaction mixtures were aliquoted into half-well 96-well plates (50 µl reactions) or full-well 96-well plates (100 µl reactions) and incubated 2 for 48 h in a KuhnerShakerX at 25°C with 75% humidity and 500 rpm with a 12.5 mm shaking diameter. The Duetz lid system (Prod. ref. CR1296, EnzyScreen BV) was used to reduce loss of liquid and to ensure even evaporation rates across the plate. The same results should be obtainable using the tubes 3 provided in the ALiCE kits and a non-humidified incubator vas per the ALiCE user manual.



Protein collection and analysis

After reaction completion, the lysate was collected and centrifuged at 15,000 x g for 10 min to clarify the solution of insoluble lysate components. Supernatants were carefully collected, and the pellet fractions were resuspended in PBS buffer in the original lysate volume. The full-lysate, pellet, and supernatant fractions were then analyzed for VLP expression using NuPAGE™ 4 to 12%, Bis-Tris (Invitrogen™) SDS-PAGE pre-cast gels and Coomassie Blue-staining. To obtain a preliminary view of VLP assembly, dot blot analysis using 1 µl of each BYL fraction spotted on an activated PVDF membrane was performed using the anti-HBc antibody 3120 (Cosmo Bio; 1:5000 dilution).

VLP purification and microscopy

For purification of HBc VLP and its variants, the CFPS reaction supernatant was heat treated for 1 h at 70°C. A centrifugation for 10 min at 15,000 x g followed, after which the supernatant was collected and processed again. Further purification was performed by hydrophobic interaction chromatography and size exclusion chromatography. Pure VLP samples were characterized via Transmission Electron Microscopy (TEM) to confirm particle assembly and integrity.

VLP immunogenicity

To assess immunogenicity of the purified HBc VLPs, dendritic cell uptake assay and human peripheral blood mononuclear cell (PBMC) stimulation assay were performed. For the dendritic cell uptake assay, recognition and endocytosis of fluorescently labeled HBc VLPs by human immature dendritic cells (iDCs) were assessed by flow cytometry and confocal microscopy. For the PBMC stimulation assay, immunogenicity was determined by measuring the release of 4 different cytokines (IL-1β, TNF-α, MCP-1 and IL-10) after stimulation with native HBc VLPs, using purified bacterial lipopolysaccharide (LPS) as a positive control.

Results and Discussion

Successful expression and assembly of HBc-VLPs in ALiCE

Using the ALiCE cell-free protein expression system, we were able to express the various HBc-VLP constructs within 48 hours. All three HBc-VLP constructs produced high protein yields at the expected sizes: 23 kDa for the Strep-fused constructs (S-HBc and HBc-S) and 21 kDa for the native HBc VLP construct. The HBc-S construct, however, showed monomers in the non-soluble fraction, indicating misfolding and aggregation. These results show that ALiCE is suitable for rapid screening of different constructs to assess not only expression, but also for correct assembly of VLPs (Figure 1).

In the subsequent dot blot assay, clear signals were observed for the HBc and S-HBc constructs, suggesting proper VLP assembly, later confirmed by TEM analysis (Figure 2). No signal was observed for the HBc-S construct, further confirming the inference from the SDS-PAGE assay, which could be due to the higher structural requirements of the spike region (Figure 1). These results demonstrate that ALiCE is a fast, high-yielding alternative to cell-based system for VLP screening, characterization, and production.

Figure 1

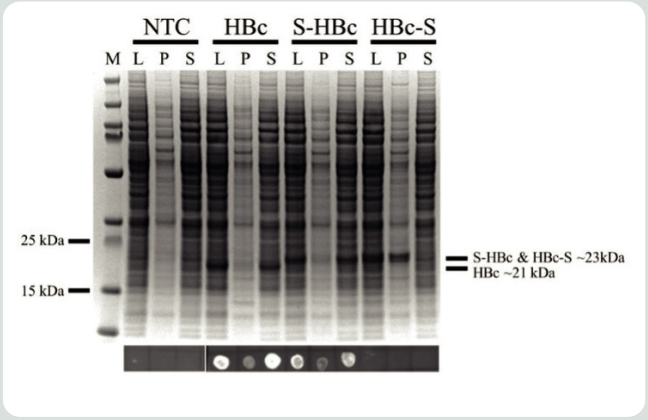


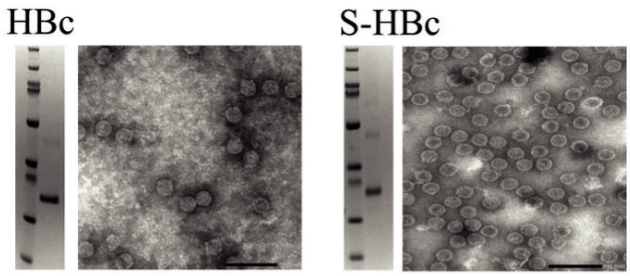
Figure 1. Successful expression and assembly of the different HBc constructs in ALiCE.

The upper panel shows SDS PAGE analysis, and the lower panel shows dot-blot analysis using a conformational antibody that binds only to correctly assembled particles.
Abbreviations. NTC: non-template control; S-HBc: hepatitis-B core antigen with N-terminal Strep tag; HBc-S: hepatitis-B core antigen with Strep tag on spike; L: lysate after reaction; P: pellet fraction after lysate centrifugation; S: supernatant fraction after lysate centrifugation

Figure 2. VLPs produced in ALiCE were fully assembled.

The SDS PAGE results after purification are shown on the left of each panel, and the corresponding TEM images are shown on the right.
Abbreviations. HBc: hepatitis-B core antigen; S-HBc: hepatitis-B core antigen with N-terminal Strep tag.

Figure 2



HBc-VLPs expressed in ALiCE produce a robust immune response

To demonstrate their applicability as putative vaccines, we showed that VLPs produced in ALiCE elicit a robust innate immune response. Using fluorescent confocal imaging, we showed that fluorescently labeled HBc VLPs were recognized and internalized by human myeloid immature dendritic cells, a crucial step in the initiation of an adaptive immune response (Figure 3A). Furthermore, HBc VLPs also triggered cytokine expression patterns consistent with a pro-inflammatory response (as observed by the positive LPS control) (Figure 3B). These results demonstrate that HBc VLPs produced in ALiCE can elicit a robust innate immune response that could lead to a protective adaptive response, overcoming a crucial barrier to producing VLP-based vaccine candidates in a cell-free system.

Figure 3

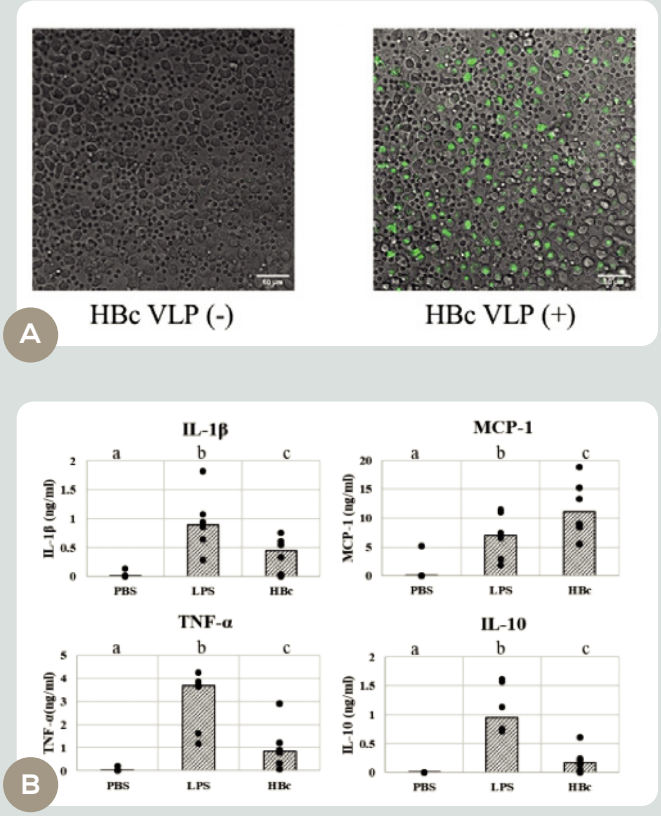


Figure 3. VLPs produced in ALiCE were recognized and internalized by human myeloid immature dendritic cells and produced a pro-inflammatory cytokine response.

A Fluorescent confocal imaging showing the recognition and endocytosis of fluorescently labeled HBc VLPs by human immature dendritic cells after incubation. Non-incubated cells (left panel) and fluorescent HBc VLP-incubated cells (right panel) are shown. B Cytokine responses of PBMCs stimulated with HBc VLPs were measured from supernatants of 6 human blood donors stimulated for 24h with either PBS (negative control), 100 ng/ml of Lipopolysaccharide (LPS; positive control) or 10 µg/ml of HBc VLP. The average for each donor (dots) and the median of all donor measurements (bars) are shown for the measured cytokines. Different letters indicate statistically significant differences between the average of each cytokine concentration for each treatment: PBS, LPS, and HBc-treated PBMCs ($p<0.01$, Wilcoxon signed-rank test)..

Conclusion

With the ALiCE cell-free protein expression system, we successfully produced and studied the assembly of several HBc VLP variants. We further demonstrated the in vitro immunogenic capabilities of the ALiCE-produced HBc VLPs, in terms of uptake by dendritic cells and cytokine response in PBMCs upon VLP stimulation. The synthesis speed, the ability to decorate carrier VLPs with antigens of interest, and the proven VLP immunogenicity make ALiCE a promising platform to rapidly screen and validate VLPs of clinical relevance, thereby accelerating their time to approval. Furthermore, this study establishes a foundation for a versatile and fast VLP-based pandemic-preparedness vaccine platform capable of rapidly adapting to new pathogens.

For full details of the study, check out our publication by Armero Gimenez et. al.

References

1. Mohsen MO, Zha L, Cabral-Miranda G, Bachmann MF. Major findings and recent advances in virus-like particle (VLP)-based vaccines. Semin Immunol. 2017 Sep 5;34:123–32.
2. Tariq H, Batool S, Asif S, Ali M, Abbasi BH. Virus-Like Particles: Revolutionary Platforms for Developing Vaccines Against Emerging Infectious Diseases. Front Microbiol. 2021;12:790121.
3. Roose K, De Baets S, Schepens B, Saelens X. Hepatitis B core-based virus-like particles to present heterologous epitopes. Expert Rev Vaccines. 2013 Feb;12(2):183–98.

Ordering information

Product	Contents	Catalog number
ALiCE® for Research - Cell-Free Protein Expression Mini Kit	6 x 50 µl ALiCE lysate, pALiCE01 and pALiCE02 vectors	AL00000001
ALiCE® for Research - Cell-Free Protein Expression Midi Kit	6x200 µl ALiCE lysate, pALiCE01 and pALiCE02 vectors	AL00000002
ALiCE® for Research - Cell-Free Protein Expression Maxi Kit	6 x 500 µl ALiCE lysate, pALiCE01 and pALiCE02 vectors	AL00000003

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