



Production of a West Nile Virus DNA Vaccine Using Perfluorosorb®S

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Introduction



In October of 2002, Aldevron was contracted by the CDC to manufacture a DNA-based West Nile (WN) Virus vaccine for use in California Condors and other endangered species. The vaccine, which is described by Davis et al. (Journal of Virology, May 2001, p. 4040-4047), consists of a 5.3kb plasmid expressing the prM and E proteins of the WN virus. The plasmid (pVAX-WN-1) is unstable in most E. coli host strains. This posed several problems as the vaccine was needed urgently.

By combining a host cell optimization, cell banking and fermentation process with the downstream purification capabilities of Perfluorosorb®S, Aldevron was able to stabilize the plasmid and manufacture bulk quantities of the vaccine. The entire process (initial development to final production) took approximately one month.

Now that a cell bank and manufacturing system is in place, large quantities of the plasmid can be produced in significantly less time.

Development

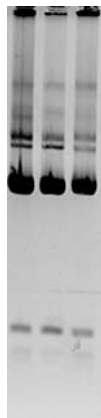


Figure 1.

pVAX-WN-1 is low yielding and unstable in most common bacterial host cells. This leads to high amounts of genomic DNA, non-supercoiled plasmid DNA, and an extra piece of DNA approximately 0.2 kb in size in routine plasmid preparations. This is unacceptable for vaccine quality DNA.



Figure 2.

Host Cell Optimization and Cell Banking.

pVAX-WN-1 was transformed into multiple E. coli K12 host strains. Many transformed colonies (from each strain) were selected and screened. The colony yielding the best combination of yield and isoform distribution was expanded into a cell bank. The cell bank was used for all pVAX-WN-1 production runs.

Production



Figure 3.

Fermentation, Lysis, and Clarification.

After determining the optimal growth conditions (media type, temperature, time, etc), fermentations were conducted using the pVAX-WN-1 cell bank as starting material. The biomass was processed with a standard alkaline lysis followed by a proprietary clarification step. The clarified lysate was subjected to an endotoxin removal step and an initial buffer exchange step.

Downstream Purification

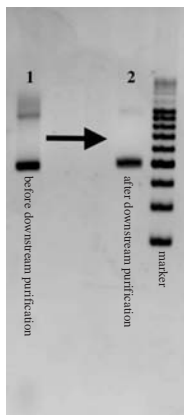
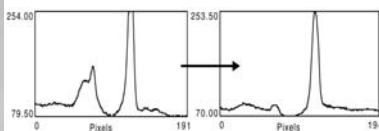


Figure 4.

Supercoil Resolution.

Perfluorosorb®S was used to resolve supercoiled pVAX-WN-1 from non-supercoiled DNA after the initial purification steps. Open circular and nicked DNA was efficiently removed from the sample as seen on the gel analysis. Plot 1 and plot 2 correspond to the first and second lanes on the gel respectively. In each of the plots, the first and second peaks correspond to non-supercoiled and supercoiled DNA respectively. Using Perfluorosorb®S, the supercoil concentration was increased to >95%. Perfluorosorb®S was also used to remove residual endotoxin, RNA, genomic DNA, and protein impurities.



Final Product

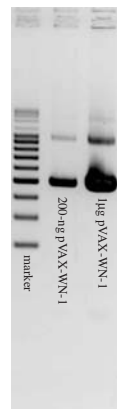


Figure 5.

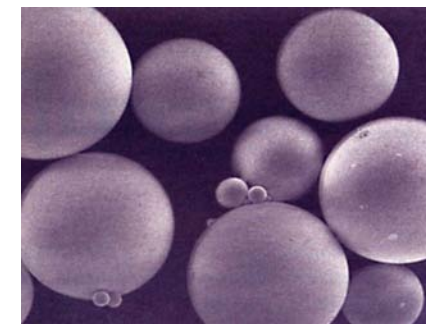
Final pVAX-WN-1 Prep.

By combining host cell selection, cell banking, optimized fermentation conditions and a downstream purification process using Perfluorosorb®S, bulk quantities of pVAX-WN-1 that meet and exceed cGMP lot release specifications can be produced. The quality of the final material is much improved when compared to early DNA preps (Figure 1). Perfluorosorb®S can be used to not only produce high-quality DNA, but to resolve supercoiled DNA.

pVAX-WN-1 Lot Release Specifications:

- Clear, colorless solution
- 2-mg/ml
- >95% supercoiled
- 260/280 >1.8, <2.0
- Proper restriction pattern
- Comigrates with reference material
- <2% gDNA
- <1% RNA
- <100 EU/mg
- <2% protein

Perfluorosorb®S



- Specifically engineered for pDNA purification
- Reverse phase chromatography
- Patented; Drug Master File
- Documented Clean in Place
- Capable of resolving supercoiled plasmid DNA
- A Product of ProMetic BioSciences