rAAV-1.3HBV for *in vivo* and *in vitro* HBV Model

General Information

Product	Cat. No.	Size	HBV Serotype
rAAV8-1.3HBV *adr*	AMV-001	1E+12vg/Vial	*adr*, wild type
rAAV8-1.3HBV *ayw*	AMV-002	1E+12vg/Vial	*ayw*, wild type
rAAV8-1.3HBV(X-) *ayw*	AMV-003	1E+12vg/Vial	*ayw*，C1397T mutation, HBx gene silence
rAAV8-1.3HBV(E-) *ayw*	AMV-004	1E+12vg/Vial	*ayw*，G1896A mutation, HBe gene silence
rAAVDJ-1.3HBV *adr*	AMV-005	1E+12vg/Vial	*adr*, wild type
rAAVDJ-1.3HBV *ayw*	AMV-006	1E+12vg/Vial	*ayw*, wild type
rAAVDJ-1.3HBV(X-) *ayw*	AMV-007	1E+12vg/Vial	*ayw*，C1397T mutation, HBx gene silence
rAAVDJ-1.3HBV(E-) *ayw*	AMV-008	1E+12vg/Vial	*ayw*，G1896A mutation, HBe gene silence

Overview

rAAV-1.3HBV is a recombinant AAV vector carrying 1.3 copies of HBV genome containing complete HBV genome and partial repeat area (Fig.1). The 1.3 copies of HBV genome can be packaged into different AAV capsids in order to meet the *in vivo* and *in vitro* modeling requirements, in which **AAV8 is commonly used for establishment of animal models and AAVDJ is used for cells transduction *in vitro*.**

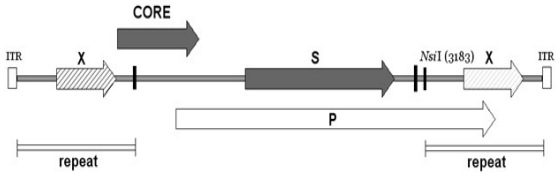

Fig.1 The map of rAAV-1.3HBV genome

In 2010, Xiaoyan Dong, et al. successfully established HBV chronic infection mouse model by transduction with rAAV8-1.3HBV *in vivo* for the first time, which lead to persistence of HBV DNA, HBeAg and HBsAg in serum for at least 10 weeks [1]. After that, HBV animal models established by rAAV8-1.3HBV were widely used in the fields of HBV pathogenic mechanism, anti-HBV drugs and vaccines development. Published data indicated that intravenous injection of rAAV8-1.3HBV established HBV infection models in C57BL/6 mice, Balb/C mice, rats and tree shrews [1-22]. However, the levels of HBV DNA, HBeAg and HBsAg in different animals are quite different. C57BL/6 mice are the most commonly used animals.

We provide rAAV-1.3HBV containing wild type or mutant HBV genome. The sequences of wild *adr* and *ayw* serotype have been submitted to GenBank. The GenBank accession number is KX449554 for *adr* serotype and KX470733 for *ayw* serotype.

rAAV-1.3HBV (X-) *ayw* is the mutant of rAAV-1.3HBV *ayw*. The C1397T mutation in X-ORF was

introduced to the repeat area of 1.3HBV genome to terminate the HBV X gene transcription (Fig.2). The mutation causes the 8th codon in the X ORF to a stop codon. HBx is required to initiate and maintain HBV replication. Contrary to cells inoculated with wild type HBV particles, cells inoculated with HBx-deficient HBV particles did not lead to productive HBV infection but comparable amounts of nuclear covalently closed circular HBV-DNA (cccDNA) were demonstrated [23-24]. Therefore, rAAV-1.3HBV (X-) *ayw* is an effective tool for HBV cccDNA epigenetics, HBV X protein function research, drug screening and drug efficacy evaluation targeting HBV cccDNA.

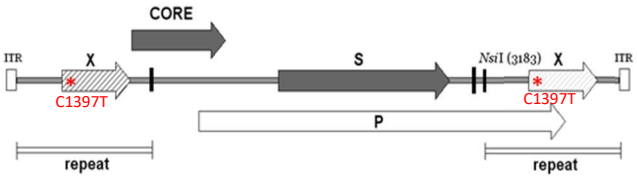

Fig.2 The map of rAAV-1.3HBV(X-) genome

rAAV-1.3HBV (E-) *ayw* is another mutant of rAAV-1.3HBV ayw, in which the G1896A mutation in HBV genome was introduced (Fig.3). The mutation causes the 28th codon in the PreC ORF to a stop codon, and the transcription of HBe gene is terminated in advance[25]. G1896A mutation is one of the most common mutations of HBV. rAAV-1.3HBV (E-) infected cells and animals don't express HBeAg. It could be used for modeling HBV G1896A mutation strain.

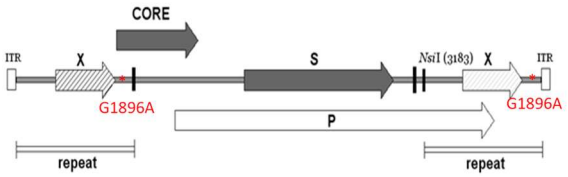

Fig.3 The map of rAAV-1.3HBV(E-) genome

Quality Control

Titer: ≥5x10^11 vg/ml (Dot blot or qPCR)
Purity: ≥85% (SDS-PAGE)
Concentration of protein: Report measured value (BCA)

Usage Guide

Handling and Storage

- rAAV stocks are supplied in liquid form at indicated titer. The storage solution is PBS with 0.001% F68.
- rAAV stocks are shipped and stored at ≤-60°C. **Please confirm that the products are frozen when received.**
- If desired, aliquot viral stock upon receipt, and store those aliquots at ≤-60°C freezer immediately.

- Once an aliquot is thawed, it can be stored at 2~8°C for a week without significant loss of biological activity.
- **Avoid repeated freezing and thawing.**

Thawing method

Thaw in 25±5°C water bath, place on ice after thawing and mix thoroughly before use.

In vivo animal use

- **rAAV8-1.3HBV was developed for establishment of HBV animal models.**
- rAAV8-1.3HBV should be administrated by intravenous injection. The recommended dosage for 4~6 weeks C57BL/6 mice is 5E+10 to 2E+11vg in 200μL volume.
- Dilute the virus with sterile PBS or dilutions supplied with products to achieve the appropriate vg number.
- The duration time and levels of HBV DNA, HBsAg and HBeAg in rAAV8-1.3HBV infected animals may change with the species and immune status of experimental animals, and is dose-dependent. **It's recommended to optimize the dosage based on the selected animals and experimental purpose.**
- Due to the slight difference in quality and quantity between different batches, it is recommended to re-optimize the dosage when using a new batch.

In vitro cell transduction

- **rAAVDJ was developed for *in vitro* cells transduction.**
- rAAVDJ-1.3HBV infects HepG2, HuH7 and primary human hepatocytes efficiently *in vitro*. The expression of HBeAg, HBsAg and HBV DNA replication usually are detective 48 hours after transduction and persistent for more than one week.
- Determine the MOI (multiplicity of infections). MOI equals number of viral genome (vg) per cell. In other words, MOI of 1 means infecting with 1 viral genome (vg) per cell. It's recommended to optimize MOI between 1E+3 to 1E+5.
- Remove the original cell culture media, and add the rAAV-containing media to cell culture. Below is a general guideline for the amount of media used:
  24-well plate: 0.2-0.3 mL/well
  12-well plate: 0.5-0.8 mL/well
  6-well plate: 2 mL/well
  60mm-plate: 3-4 mL/plate
  10cm-plate: 8-12 mL/plate
- Adding 5 to 20mM sodium butyrate to culture medium 2~8h after transduction usually enhances the gene expression.
- Due to the slight difference in quality and quantity between different batches, it is recommended to re-optimize the dosage when using a new batch.

Biosafety Considerations

rAAV-1.3HBV products are potentially infectious and pathogenic to humans and mammals, and relevant experimental operations must be carried out in the BSL-2 or ABSL-2 laboratory. Operators should have been vaccinated with hepatitis B vaccine, acquired protective immunity, and have completed professional biosafety training before operations. The disposal of waste strictly complies with the BSL-2 or ABSL-2 guidelines.

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