

# User Manual Viruguard

## Directory

Directory.....	1
Safe Use of AAV.....	2
Storage and transportation of Viruguard.....	2
Dilution and administration of Viruguard.....	2
Characteristics of Viruguard.....	3
Analysis of Viruguard.....	3
Contact us.....	4

## Safe Use of Viruguard

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1. Adenovirus related experiments can only be conducted in biosafety level 2 facilities (BL-2 level).
2. Please equip with appropriate with PPE including lab coat, face shield, and gloves, no skin should be exposed during experiment and your operation.
3. Take caution when operating related experiments. If virus suspension is splashed, or biosafety cabinet is contaminated with virus during operation, the table-board should be scrubbed by 75% ethanol and SDS to keep you safe and protect those who may operate with the cabinet. All the consumable items or apparatus, including but not limited to centrifuge tube, pipette tips, culture plates, glass containers contacting virus must be soaked in disinfectant before disposal or recycling.
4. Seal the tube with parafilm when centrifuging to avoid possible leaking.
5. Associated waste materials contaminated by Viruguard need to be specially collected and processed according to your regional medical waste disposal law.
6. Hand hygiene and cleaning should be carefully followed.

## Storage and transportation of Viruguard

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Viruguard can be stored at 4°C for less than a week before using, long term storage at 4°C is not recommended since high temperature can lead to denaturalization. Aliquot bottled viral stock should be kept at liquid nitrogen or -80°C freezer during transportation and storage to ensure its treatment effect until expiration date. Viral titer redetection are highly recommended if Viruguard has been stored for 12 months.

## Dilution and administration of Viruguard

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Virus should be dissolved bathed in ice or under 4°C with PBS and saline dissolution, or specific medium to the titer which will be determined by modelling, animal studies and clinical trials in the near future. Intratumoral injection are indicated to have better function according to our model. Experienced and qualified doctors or nurses are required for the injection. Severe complications including haemorrhage, mis-injection into portal vein or hepatic artery, allergic shock should be urgently processed symptomatically by intervention therapy, internal medicine treatment and even surgery.

### **Tips**

- Avoid Viruguard exposure to extreme conditions including unsuitable pH, temperature, chelating reagents, and chemicals.
- If respiratory symptoms, skin erythema and related symptoms occur after operating with Viruguard or administration, please contact your GP or go to the local hospital immediately.
- Avoid repeated freezing and thawing to prevent expiration.

- Avoid diluting Viruguard in salt dilution that is not mentioned above. Viruguard can possibly precipitate or aggregate in unfavorable solution.

## Characteristics of Viruguard

- High efficiency. The immunogenicity of viruguard has been quickly reduced by the design of TLR9i. And a certain titre will be further discovered to ensure the efficiency.
- Tissue Tropism. With the design of anti-GPC3 scfv and miRNA sponge, siRNA under the control of HCC-specific promoter, the Viruguard possesses the incomparable ability to target hepatic cancer cells.
- High quality. The manufacture of viruguard are subjected to rigorous testing, including mycoplasma and endotoxin test.
- High safety level. The safety of Viruguard are considered through the design of fibre-fused scfv, specific killing and replication

## Analysis of Viruguard

Certificate of Analysis		
Name	Viruguard	
Vector name	YOE-AV001-insert DNA	
Manufacture No.	AV211018D01	
Storage	-80°C storage, 12 months	
The quality of Viruguard		
Test	Quality standard	Test results
Viral titre	> 10 <sup>10</sup> PFU	3.49E+10
Bacteria	Negative	Negative
Mycoplasma	Negative	Negative
Note:		
1. Titre measurement		
The titre of virus was measured by TCID50. The virus stock was gradiently diluted to the concentration from 10 <sup>-1</sup> to 10 <sup>-10</sup> . From the max concentration, 100 µl solution of every gradient of virus 100 µl was inoculated to the HEK293A 96-well plate with wells numbered 1 to 10. CPE was observed under the inverted microscope after 10 days.		
2. Bacteria test		
Adenovirus stock was inoculated into growth medium to perform bacteria test.		

### 3. Mycoplasma test

Adenovirus sample was inoculated to the marker cell, and Hoechst 33259 was used to dye the DNA. The mycoplasma was indicated as dotted fluorescence around the nucleus of marker cells.

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