User Instructions:

Herpes Simplex Virus (HSV) for In Vivo Applications

Content

The table below shows the components associated with the custom HSV. The virus titer is specified in the certificate of analysis (COA) document.

Scale	Deliverable	Specification	Recommended Use
Pilot-scale packaging and ultra-purification	Custom virus	$>10^7$ PFU/ml, 10x100 ul	In vivo applications
Medium-scale packaging and ultra-purification	Custom virus	$>10^8$ PFU/ml, 10x100 ul	In vivo applications

Storage and Handling

1. VectorBuilder's ultra-purified HSV is recommended for in vivo applications. Our ultra-purified HSV is stored in PBS-based buffer containing sucrose and is shipped on dry ice.
2. Upon receiving, HSV should be stored at -80°C for the long term (stable for at least 6 months), or -20°C for use within one week. The shelf life for HSV is approximately one year.
3. Please avoid repeated freeze-thaw cycles of HSV, as this can result in a large titer drop.

Safety Precautions

HSV BACYAC vectors generated by VectorBuilder cover HSV-1 wildtypes strain KOS. Wildtype HSV-1 can replicate in vivo and may cause death in rodents, particularly in those that are immunocompromised or having ineffective immune responses. Additionally, we offer replication defective HSV with deletions/mutations in genes essential for viral replication, and attenuated HSV that carries deletions/mutations in non-essential genes, as well as other customized mutagenesis services. HSV is typically handled in a BSL2 facility. However, biosafety policies can vary considerably from one institution to another. Therefore, it is the responsibility of the researchers to handle all viral vectors following appropriate biosafety guidelines that apply for their institution.

Recommended Protocol for Infection

Various methods can be applied to infect rodents with HSV, depending on the experimental goals and the area of study. Common infectious routes include systemic infection via intraperitoneal (IP) injection [1], and intravenous (IV) injection, as well as ocular infection [2], footpad inoculation [3], intracerebral or intracerebroventricular inoculation [4], etc.

Ocular Infection

The ocular infection model of HSV-1 in mice has been employed to study viral pathogenesis, immune response, and viral latency in tissues, including neurons in the brain and sensory ganglia [5][6].

Materials

- Adult mice, recommended C57BL/6N line, 6-8 weeks of age
- Ultra-purified HSV-1 (recommended dose is 10^5-10^6 PFU)
- 70% ethanol
- Anesthetics and analgesics (e.g. sodium pentobarbital or ketamine)
- 28 gauge needle

Procedures

1. **Anesthesia**

    - C57BL/6 (6-8 weeks) mice can be anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg of body weight), prior to inoculation. Alternatively, mice can be anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (12 mg/kg).

      Note: Follow the institution's guidelines for the anesthesia procedure. Ensure that you use the specified drug and dose as recommended.

    - The animal should reach deep anesthesia within ~10 min. Check the lack of response to nociceptive stimuli to confirm depth of anesthesia by pinching the tail.

2. **Cornea scarification**

    - Gently hold the eyelid of anesthetized mouse to expose the cornea.
    - Using a sterile 28-gauge needle, make small, superficial scratches on the corneal surface. Be careful to avoid penetrating too deeply and damaging the underlying structures.
    - Ensure the scratches cover the entire corneal surface to facilitate uniform viral infection.

      Note: Place the anesthetized mouse under a dissecting microscope for better visibility. Use sterile instruments and maintain a clean working environment to prevent contamination.

3. **Infection**

    - Apply a small volume (usually 5-10 µL) of HSV-1 directly onto the surface of scarified cornea. Allow the virus to remain in contact with the cornea for a few minutes to ensure proper absorption.

Validation

Acute HSV-1 expression is typically observed 3-7 days post-infection. Latent virus expression in neurons, particularly in the trigeminal ganglia, is typically observed approximately 4 weeks post-infection. This time frame allows the virus to establish latency within the neurons following the initial acute phase of infection. The exact timing can vary depending on factors such as the viral strain, the host's immune response, and specific experimental conditions. Health conditions of the infected mice should be monitored continuously throughout the process.

Tail Vein Injection

Intravenous (IV) injection via the lateral tail vein allows modeling of systemic infection and studying of viral pathogenesis. In the example below, an immune-compromised mouse strain is used.

Mouse strain and dose

- Adult mice, recommended male NBSGW (JAX #026497) line, at least 4 weeks of age
- Ultra-purified HSV-1 (recommended dose is 1.2x109 PFU/kg of body weight)
- 70% ethanol
- Heat source
- Mouse restrainer
- Cotton gauze pads
- 0.5 mL syringe with 27 gauge ½ inch needle

Procedures

1. **Physical restraint**
    - Mice can be physically restrained using a commercially available restraining device. Gently introduce the mouse to be injected into restrainer making sure the tail is exposed for further manipulations.
2. **Vasodilation**
    - To induce peripheral vasodilation, warm the mouse by either dipping the tail in warm water (43°C) or placing the animal near a heat lamp for 5 to 10 minutes prior to injection.

      Note: When using a heat lamp, place the lamp about 15 to 25 cm above the cage and observe the mice for 5 minutes. Stop heating once the mice huddle together in one corner of the cage.
3. **Injection**
    - Making sure the mouse is placed in the restraining device in a stern position, slightly rotate the tail to visualize the lateral vein. Disinfect the injection site with 70% ethanol and wipe off excess alcohol with gauze pad.
    - Using a 0.5 ml syringe with a 27 gauge needle, draw up at least 0.1 ml of ultra-purified HSV (dilute HSV to the correspoding dose in advance). Carefully remove air from the needle.
    - Grasp the distal part of the tail and twist it slightly to the lateral vein's side (**Figure 1**). Insert the needle at a shallow angle (as the tail vein is relatively close to the surface) and inject diluted HSV into the vein. The injection should proceed smoothly without any resistance if the needle is correctly positioned, and the vein will transiently shift to a clear color. Stop injecting if any local swelling is observed, as in that case the virus is being injected into the tail tissue, not the vein.

      Note: Start your injection from the lower portion of the tail about 1/3 from the tip. This allows you to move up the tail if the previous injection was unsuccessful.

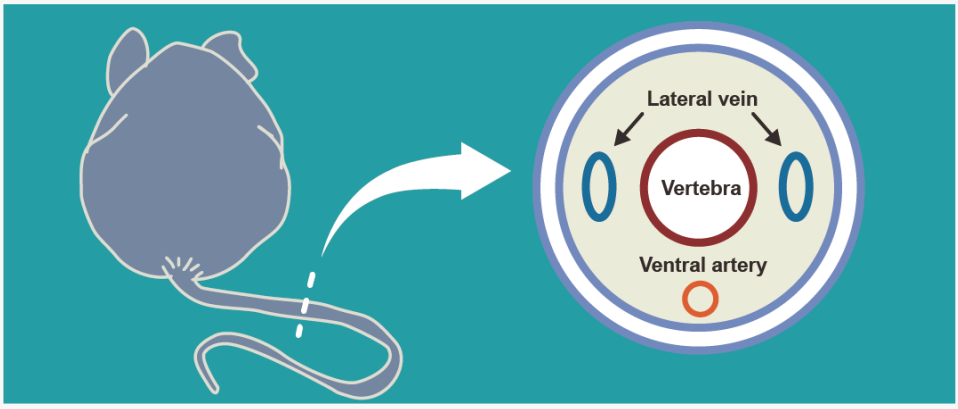

Figure 1. Diagram of a transverse sectional view of mouse tail lateral veins and ventral artery.

Validation

To determine the efficiency of in vivo transduction, mice can be sacrificed at day 7 after virus injection. Mice may exhibit hind limb motor impairment by collection time if using an immune-compromised line.

Anticipated Results

An example of successful in vivo transduction shown in **Figure 2**.

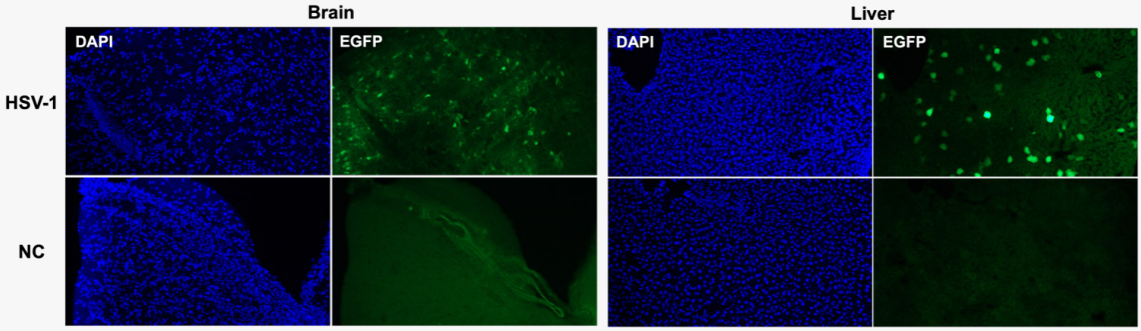

Figure 2. HSV-1 expressing EGFP was used for IV injection into NBSGW mice. Seven days post-injection, cryo-sectioned brain and liver tissues were imaged for EGFP expression. Green: EGFP. Blue: DAPI.

References

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3. Devi-Rao, G. B., et al. "Herpes simplex virus type 1 DNA replication and gene expression during explant-induced reactivation of latently infected murine sensory ganglia." Journal of virology 68.3 (1994): 1271-1282.
4. Sundaresan, Periasamy, et al. "Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation in mice." Journal of virology 74.8 (2000): 3832-3841.
5. Doll, Jessica R., et al. "Resolution of herpes simplex virus reactivation in vivo results in neuronal destruction." PLoS pathogens 16.3 (2020): e1008296.
6. Sawtell, N. M., et al. "The latent herpes simplex virus type 1 genome copy number in individual neurons is virus strain specific and correlates with reactivation." Journal of virology 72.7 (1998): 5343-5350.