User Instructions:
Herpes Simplex Virus (HSV) for In Vitro Applications

Storage and Handling

1. Our non-purified HSV is stored in HBSS buffer 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 can cover HSV-1 wildtypes strain. 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, and 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 Transduction

1. Day before transduction (Day 0)

   Plate target cells in the appropriate medium so that they will be 60-70% confluent at the time of transduction. Incubate for 18-20 hours at 37°C in a humidified 5% CO_2 incubator. When using BHK-21 cells, refer to the recommended confluency at transduction as illustrated in **Figure 1** below.

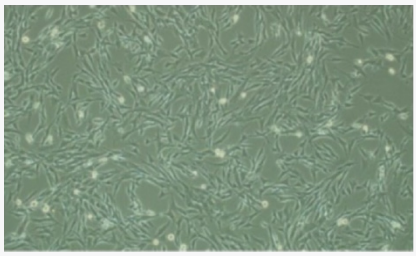

Figure 1. BHK-21 cells at the time of HSV transduction (bright field). Magnification: 100x.

2. Day of transduction (Day 1)

   - Thaw the virus on ice. Occasionally, the content may appear slightly cloudy, which is normal. Mix the virus gently, take an appropriate amount of virus as needed to achieve the desired MOI, place in an appropriate amount of medium, and mix gently (but do not vortex).

     Note: Start infecting the cells at an MOI between 0.01 and 0.05 if cells are readily infected such as BHK-21. For some cell lines, a higher MOI may be needed.

   - Aspirate old medium from target cells, then add the virus-containing medium onto the cells.
   - Swirl the plate gently to mix and incubate at 37°C in a humidified 5% CO_2 incubator overnight.

3. Later Day 1, or Day 2

   Remove the virus-containing medium and replace it with fresh complete culture medium. Incubate at 37°C in a humidified 5% CO_2 incubator.

   Note: The incubation time of HSV is generally 6-12 hours. Certain cell lines may require longer incubation.

   Note: If the virus genome encodes a fluorescent marker, it may be visible 12 hours post-transduction. In most cases, the fluorescent signal will become stable 24-48 hours post-transduction.

Example of a Successful Transduction with HSV

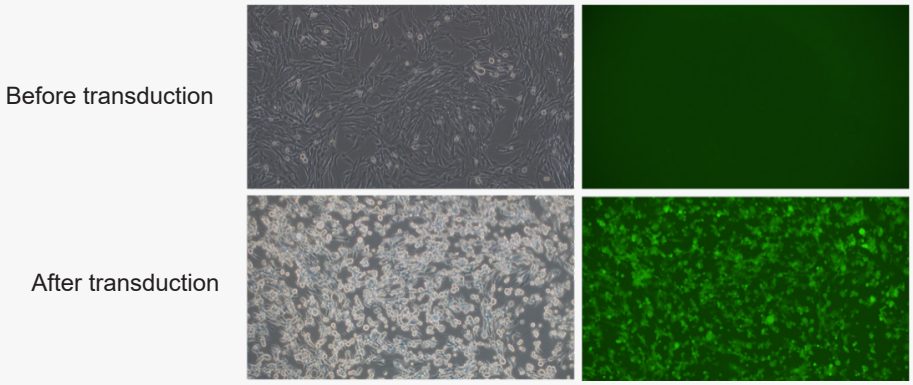

Figure 2. HSV expressing EGFP was used for transducing BHK-21 cells. Images were taken before transduction and at 48 hours post-transduction at MOI of 0.05. Magnification: 100x. 50 ms. Left: bright field. Right: EGFP.