User Instructions:
Vesicular Stomatitis Virus (VSV) for In Vitro Applications

Content

The following tables show the components associated with custom VSV. The virus titer is given in the certificate of analysis (COA) document.

Scale	Deliverable	Specifications	Recommended Use
Medium-scale packaging	Custom virus	Concentrated virus (>10^7 PFU/ml, 10x100 ul)	Cell culture applications
	Control virus (VSV pseudotyped with VSV-G)	Concentrated virus (>10^7 PFU/ml, 2X100 ul)	
Large-scale packaging	Custom virus	Concentrated virus (>10^8 PFU/ml, 10x100 ul)	
	Control virus (VSV pseudotyped with VSV-G)	Concentrated virus (>10^8 PFU/ml, 2X100 ul)	

Storage and Handling

1. VectorBuilder's non-ultra-purified VSV is recommended for in vitro applications. Our VSV is stored in the HBSS buffer and is shipped on dry ice.
2. Upon receiving, it 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 VSV is approximately one year.
3. Please avoid repeated freeze-thaw cycles of VSV, as this can result in a large titer drop.

Safety Precautions

Recombinant VSV vectors generated by VectorBuilder are deficient in replication due to the deletion of the envelope glycoprotein G gene. The envelope proteins of heterologous viruses, including viruses requiring high-level containment can be supplied in trans for producing VSV pseudotypes. Since VSV pseudotypes are unable to undergo more than a single round of replication, they can be safely handled in a regular BSL2 facility. This makes VSV vectors highly suitable for studying cell entry mechanisms of high-risk viruses without the requirement of high-level containment.

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.

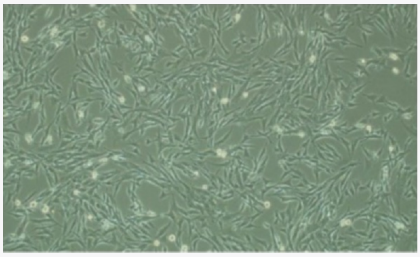

Figure 1. BHK-21 cells at the time of VSV transduction (bright field). Magnification: 100x.

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 the 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). To maximize transduction efficiency, use the minimum amount of medium necessary to cover the surface of the plate. Typically, this means ~100 µl/cm^2. For example, when transduction is performed in 6-well plates, we recommend 1 ml of medium per well given that the growth area per well is ~10 cm^2.

     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 incubator overnight.

3. 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: If the virus genome encodes a fluorescent marker, weak fluorescence may become visible 5-6 hours post-transduction in cells with strong metabolic capabilities, such as BHK-21 cells. In most cases, the fluorescent signal will become stable 24-48 hours post-transduction.

Example of a Successful Transduction of VSV Pseudotyped with VSV-G Protein

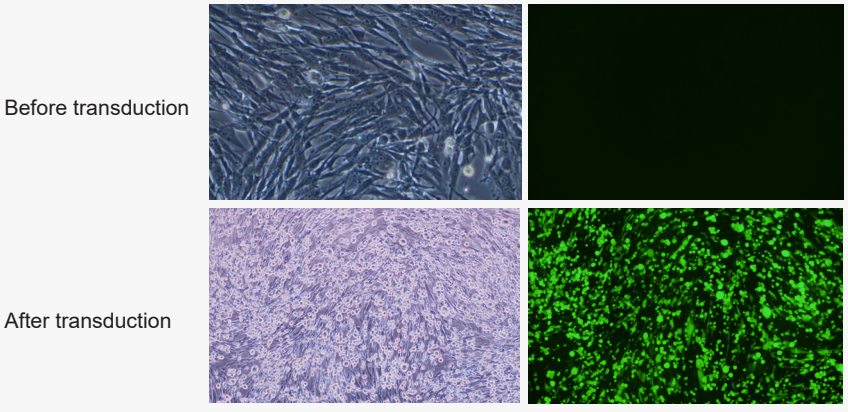

Figure 2. VSV expressing EGFP and pseudotyped with VSV-G protein was used for transducing BHK-21 cells. Images were taken before transduction and at 24 hours post-transduction. Magnification: 100x. Left: bright field. Right: EGFP.