User Instructions: VACV for In Vitro Applications

Content

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

Scale	Deliverable	Specification	Recommended use
Pilot-scale packaging	Custom virus	>10^8 PFU/ml, 10×25 µl	Cell culture applications
Medium-scale packaging	Custom virus	>10^8 PFU/ml, 10×100 µl	
Ultra-purified medium-scale packaging	Custom virus	>10^8 PFU/ml, 10×100 µl	

Storage and Handling

1. VectorBuilder's VACV is preserved in HBSS buffer and is shipped with dry ice. Upon receiving, it should be stored at -80°C for long-term storage (stable for at least 6 months), or -20°C if intended for use within one week.
2. The shelf life of VACV is approximately one year.
3. Please avoid repeated freeze-thaw cycles of VACV, as this can lead to a significant titer drop.

Safety Precautions

All VACV vectors from VectorBuilder are not able to generate live virus directly. However, they can be transfected into packaging cells that are infected with non-replicating fowlpox virus for live viral production. VectorBuilder has meticulously optimized this packaging process for high titers produced safely and efficiently. **We strongly recommend that the virus should be handled according to Biosafety Level 2 (BSL-2) criteria.** All procedures related to the handling, storage, and disposal of biohazardous wastes must strictly adhere to both published and institutional criteria. Caution must be exercised during the production and manipulation of recombinant VACV.

Recommended Protocol for Transduction

We recommend using the EGFP-expressing control VACV to determine the optimal multiplicity of infection (MOI) for your target cells. MOI is defined as the ratio of the number of viral particles to the number of the host cells. In other words, an MOI of 1 refers to one host cell for one viral particle.

Protocol for transducing mammalian cell line:

1. Day before transduction (Day 0)

   Plate the target cells in appropriate media so that they will be near 90% confluency at the time of transduction. Incubate the plates for 18-20 hours at 37°C in a humidified 5% CO_2 incubator.

2. Day of transduction (Day 1)

   - Thaw the virus on ice. Take the appropriate amount of virus required to achieve the desired MOI, place in an appropriate amount of medium and mix gently, avoid vortexing. To maximize transduction efficiency, use the minimum amount of medium required to cover the surface of the plate. For example, when the transduction is performed in 6-well plates, it is common to use 1 ml of culture medium per well.

     Note: Start infecting the cells at MOI of 0.01-0.05 if cells are readily infected. For some cell lines, a higher MOI might be needed.

   - Remove the old medium from the target cells and replace with the virus-containing medium.
   - Gently swirl the plate to ensure thorough mixing and coverage of the cells. Incubate overnight at 37°C in a humidified 5% CO_2 incubator.

     Note: If there are concerns about the potential adverse effects of exposure to the viral supernatant on the target cells, it is advisable to limit the transduction duration to 2-4 hours.

3. Day 2

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

4. Day 3 and onward

   Analyze gene expression at the desired time points following viral infection. In general, detectable levels of your gene product should become evident within 24-48 hours after transduction.

   Note: In actively dividing cells (i.e. doubling time of approximately 24 hours), transgene expression is generally detectable within 24 hours of transduction, with maximal expression observed at 48-96 hours (2-4 days) post transduction. Expression levels generally start to decline 5 days post-transduction. In cell lines that exhibit longer doubling times or non-dividing cell lines, high levels of transgene expression normally persist for a longer time. If you are transducing VACV into your mammalian cell line for the first time, we recommend performing a time course study to determine the optimal temporal conditions for expression of your transgene.

Example of a Successful Transduction

An example of successful transduction is shown in **Figure 1**.

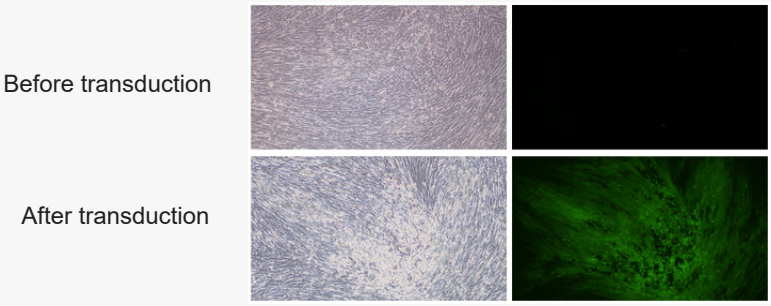

Figure 1. BHK21 cells were transduced with VACV produced from VectorBuilder BACYAC vector carrying an EGFP reporter at an MOI of 0.05. Images were taken before transduction and 24 hours post-transduction. Magnification: 100x. Left: bright field. Right: EGFP.