User Instructions: Baculovirus for In Vitro Applications

Content

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

Content	Deliverable	Specification	Recommended use
Medium-scale packaging	Custom virus	Concentrated virus (>1×10^6 PFU/ml, 10×100 ul, DPBS buffer)	Cell culture
	Control virus	Concentrated virus (>1×10^6 PFU/ml, 2×100 ul, DPBS buffer)	
Large-scale packaging	Custom virus	Concentrated virus (>1×10^7 PFU/ml, 10×100 ul, DPBS buffer)	Cell culture
	Control virus	Concentrated virus (>1×10^6 PFU/ml, 2×100 ul, DPBS buffer)	

Storage and Handling

1. Upon receiving, baculovirus should be stored at -80°C for long term storage (stable for at least 1 year), or -20°C for short term storage (stable for 2~3 weeks).
2. Thaw the vial of baculovirus on ice prior to use and keep it on ice during the experiment. Thawed baculovirus can be stored at 4°C for 1~2 weeks without significant loss of biological activity.
3. After thawing, baculovirus can be dispensed into smaller aliquots according to the quantity used in your experiment and then refrozen. If you need to dilute the virus, you may use DPBS, but do so ONLY immediately prior to use.

CAUTION: Do not freeze and thaw your baculovirus sample multiple times. Please avoid repeated freeze-thaw cycles of baculovirus, as this can result in a large titer drop.

Safety Precautions

All baculovirus from VectorBuilder cannot replicate outside of insect cells and are nonpathogenic to mammals and plants. The virus, however, still pose a biohazard risk in theory because it can transduce primary human cells. We recommend that the virus should be handled according to Biosafety Level 2 (BSL-2) criteria. All handling, storage and disposal of biohazard waste must be in accordance with published and institutional criteria.

Transduction of Target Cells

We recommend using the EGFP-expressing control baculovirus to determine the optimal multiplicity of infection (MOI) for your target cells. MOI is defined as the number of infectious viral particles per cell. In other words, an MOI of 1 refers to using 1 plaque forming unit (PFU) per cell.

Protocol for transducing Sf9 insect cells in suspension culture for pilot study

1. Seeding cells (Day 0)

   Seed target cells in appropriate serum-free medium (e.g. II SFM) and grow the cells in culture flasks for two days until they attain the cell density of 1.0-1.5x10^6 cells/ml.

2. Transduction (Day 2)

- Thaw virus on ice. Take the appropriate amount of virus as needed to achieve the desired MOI (our recommended MOI is 5~10), add the virus to an appropriate amount of medium, and mix gently (but do not vortex).
- Centrifuge down the target cells at 900 rpm and aspirate the old medium. Add the virus-containing medium onto the cells.

Note: To maximize transduction efficiency, the volume of virus-containing medium should be reduced to the minimum amount that necessary to suspend cells. For example, VectorBuilder use 12.5 ml of virus-containing medium to infect cells in a T125 flask.

- Swirl the flask gently to mix well. Incubate the culture at 27 °C, 110 rpm with the relative humility of 95%.

3. Day 3 and onward

   To confirm the progress of infection, observe morphologies and cell densities every 24 hours after transduction.

Note: Optimal transgene protein expression is often between 48 and 72 hours post-infection, so it is recommended to sample cultures every 8 to 12 hours after 24 hours post-infection. Importantly, replenishing additional amount of medium could also improve protein expression efficiency.

4. Cell pellet/supernatant storage: For non-secreted protein, cell pellet harvested from a T125 flask could be suspended in 500 ul medium and store at -80°C. Regarding secreted proteins, the clarified supernatants could be stored at 4°C for 1-2 days.
5. Assay your cell pellets or supernatant for recombinant product yields and/or activity. Determine the optimal harvest time for your protein production.
6. Scale up the protein production according to the optimal parameters of MOI and harvest time.