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## **VSV Pseudovirus Safety Statement**

### **1) Background of VSV virus:**

Vesicular stomatitis virus (VSV) is a prototypic animal virus, a member of the Vesiculovirus genus in the family Rhabdoviridae. Wild type (WT) VSV transmission most occurs in animals through insect bites. Human VSV transmission is rare with much less severe disease symptoms, alike a mild flu-like illness or asymptomatic (Ref 1-2). VSV has eight main serotypes: Indiana, New Jersey, Cocal, Alagoas, Isfahan, Chandipura, Maraba, and Piry.

VSV is a bullet-shaped, enveloped virus consisting of a single strand of negative-sense RNA, which encodes five major proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and large protein (L). The simplicity of VSV genome enables its extensively application in research field. Besides, the replication of wild type VSV does not incorporate into host genome, unlike HIV or lentivector, therefore, its application is much safer. In the laboratory, VSV has been engineered to target cancer cells or to stimulate immunity against diseases such as AIDS or influenza (Ref 3). Moreover, based on VSV backbone, ERVEBO (rVSV $\Delta$ G-ZEBOV-GP) Ebola vaccine has been approved to use in human by the FDA in 2019.

The assessment of appropriate containment for construction and laboratory use of WT VSV (except strains of Chandipura and Piry) for BSL2 is based on the 1999 CDC/NIH BMBL. WT VSV has been treated as a BSL2 pathogen widely by scholars (ref 4) and commercial providers, such as ATCC (Catalog: VR-1238).

## **2) Bio-Safety statement about ReVacc Scientific's VSV pseudovirus products:**

Virus entry is one of the most important studies to assess the efficacy of vaccine, therapeutic medicine and antibody. Pseudovirus provides the opportunity for researchers to conduct such study in BSL2 labs, compare with authentic virus that has to be handled in higher level biocontainment. The high-maintenance and limit of BSL3/4 facilities significantly slows down the related studies, such COVID-19 vaccine research.

Engineered recombinant VSV (rVSV) that glycoprotein (G) gene was deleted provides solution for heterologous pseudovirus (fake virus) of these viruses that require high-level biocontainment, such as SARS-CoV-2 (BSL3 pathogen, virus causing COVID-19), Ebola or Marburg (BSL4 pathogen) viruses. With more potency than lentivirus pseudovirus, VSV-pseudovirus provides a robust platform for high-throughput virus entry study to screen anti-viral medicines or immune-sera.

ReVacc Scientific's VSV pseudovirus product is engineered based BSL2 VSV strain. It only generates replication-incompetent VSV pseudovirus with envelope protein from heterologous viruses, including viruses that require high-level biocontainment, such as SARS-CoV-2, Ebola and Marburg viruses. The most important precaution to prevent replication-competent virus generation is to eliminate the envelope gene in final VSV-pseudovirus by using two steps of production, different from lentivirus backbone-based pseudovirus that is produced by co-transfection of envelope gene with packaging genes. Besides, the final products are centrifuged and filtered (0.45  $\mu\text{m}$ ) to remove cells or debris that might contain vectors. The last but not least, our routine and quality control procedures including pseudovirus titration and neutralization verification with positive control monoclonal antibodies minimize the likelihood of contamination with any envelope genes in our final products.

## **3) VSV pseudovirus Handling:**

Even VSV-pseudovirus is much safer than WT VSV, we suggest that researchers handle VSV-pseudovirus under BSL2 containment. We request any buyers who intend to purchase any

pseudovirus products to fill a form (page 2 of New Customer Application form) to meet the requirement for BSL2, covering the training knowledge, equipment and facility. Briefly, lab coat, gloves, eye protection are required to handle pseudovirus products in a biosafety cabinet. Contacted tips & tubes should be decontaminated by 10% disinfecting bleach and further autoclaved. We suggest that buyers should consult with own institution's biosafety officer for specific instructions.

In case of spills: Allow aerosols to settle and, wearing protective clothing/eye protection, gently cover spill with paper towels and apply appropriate disinfectant (such as 10% disinfecting bleach), starting at the perimeter and working towards the center. Allow sufficient contact time before clean-up (30 min) (ref 5).

For disposal: Decontaminate all materials for disposal by chemical disinfection (such as 10% disinfecting bleach) prior to autoclave (ref 5).

Storage: In sealed, leak-proof containers that are appropriately labelled and locked in a -80°C freezer.

## **References:**

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