

# VV<sub>(Li)</sub>-RlucGFP-CBG-RFP-GusA

## Product Description

Product Name: VV<sub>(Li)</sub>-RlucGFP-CBG-RFP-GusA  
(Standard Titer, 0.25 mL)  
Catalog Number: OV4003  
Lot Number: OV-IM14

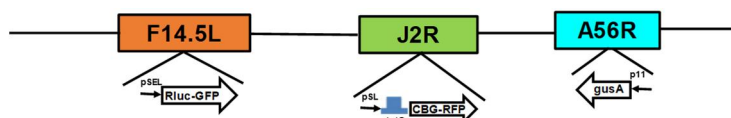
Shipping conditions: Dry ice  
Storage conditions: Store at ≤ -70°C upon receipt, avoid subjecting to multiple freeze-thaw cycles

Species: Vaccinia  
Virus strain: Lister (Li)  
Transgenes: Red fluorescence protein (RFP)  
Click beetle green luciferase (CBG)  
Green fluorescent protein (GFP)  
β-glucuronidase (GusA)  
Renilla luciferase (Rluc)

Production Cell Line: Vero  
Storage Media: 1mM TrisCl, pH 9  
Propagation: Inoculate producer cells (e.g. Vero) at an MOI of 0.1 for 2-3 hours. After 48 hours harvest culture supernatant and cells and freeze-thaw or sonicate 3X. Clarify the supernatant by low-speed centrifugation and purify through a sucrose cushion. Resuspend the viral pellet in 1mM TrisCl, pH 9.

VV<sub>(Li)</sub>-RlucGFP-CBG-RFP-GusA is a recombinant vaccinia virus (VV) encoding the following additional transcription units: 1) the Renilla luciferase (Rluc)-Aequorea green fluorescent protein (GFP) fusion cDNA under transcriptional control of the VV synthetic early late (pSEL) promoter, 2) the tet-operator sequence *tetO* followed by click beetle green luciferase (CBG)-red fluorescence protein (RFP) fusion cDNA under transcriptional control of the VV synthetic late (pSL) promoter, and 3) the β-glucuronidase (GusA) cDNA under transcriptional control of p11 promoter. These additional transcription units are inserted into, and disrupt the function of, the viral F14.5L, J2R (thymidine kinase), and A56R (hemagglutinin) genes, respectively (see diagram below). The purchased virus is ready for use in cell-killing assays or can be amplified to create additional virus stocks.

## Genome:



## References:

Kirscher et al., *Theranostics*, 2015, 5(10):1045-1057  
Zhang et al. *Cancer Res* 2007, 67:10038-10046  
Stritzker et al., *J. Virol.* 2014, 88: 11556-11567  
Duggal et al., *J. Transl. Med.* 2013, 11:155.  
Puhlmann, et al., *Cancer Gene Therapy*, 2000 7, 66–73  
Gholami et al. *Breast Cancer Research*, 2013, 15: R26

## Safety Precaution:

All culture work with Vaccinia should be performed by trained personnel and performed under BSL2 containment following NIH guidelines.

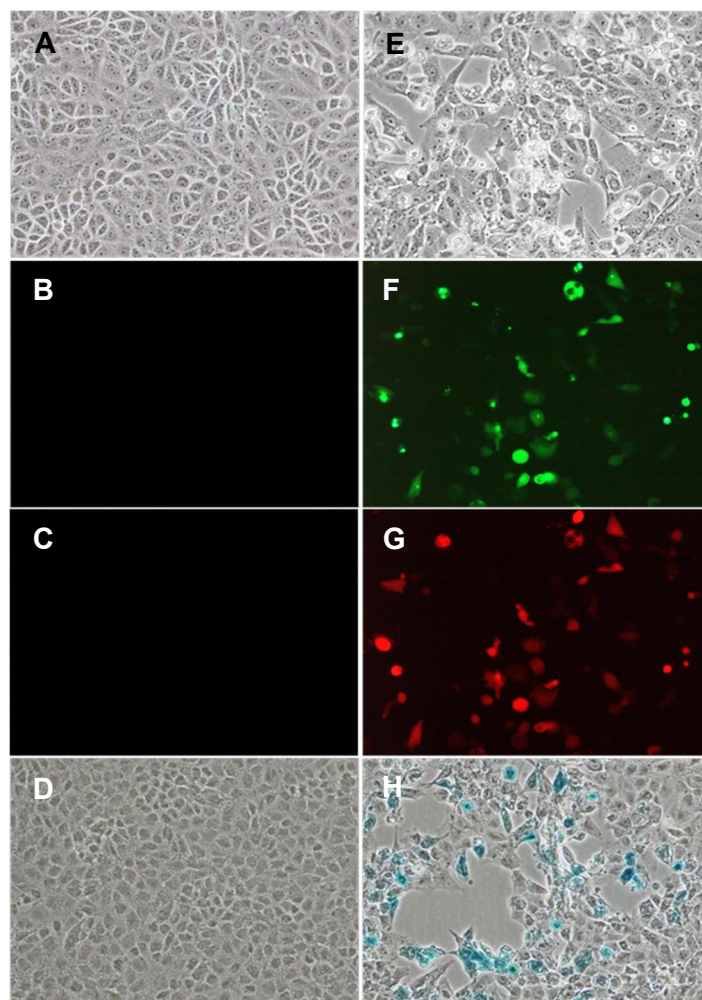


## Certificate of Analysis

Testing performed by Imanis Life Sciences

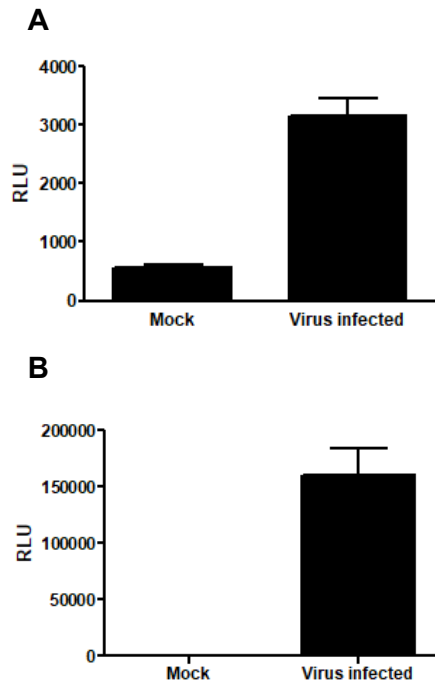
Test description	Result
Titer (on Vero)	7.70x10 <sup>7</sup> TCID <sub>50</sub> units/mL
Sterility	Pass QC
Cytopathic Effect	Pass QC
Endotoxin Test	Pass QC
Fluorescence Expression	Pass QC
Luciferase Expression	Pass QC

## Infectivity



A-D: Mock-infected Vero cells.  
E-H: VV<sub>(Li)</sub>-RlucGFP-CBG-RFP-GusA infected Vero cells (MOI 0.1) at 48 h.p.i.  
D&H: β-glucuronidase activity: Cells were incubated with media containing X-Gluc (5-bromo-4-chloro-3-indolyl-beta-glucuronide).

## Luciferase Expression



Vero cells were mock-infected or infected with VV<sub>(Li)</sub>-RlucGFP-CBG-RFP-GusA at an MOI of 1. After 48 hours, 1.25 µg of Renilla luciferase substrate coelenterazine (A) or Click beetle luciferase substrate d-luciferin (B) was added to the wells and luminescence (RLU) was immediately measured using a microplate reader.

**Quality Control by: MT**  
**Quality Assurance by: RLV**  
**Effective Date: 17-Nov-2017**

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