$VV_{(Li)}$-iCBG-RFP-GusA

Product Description

Product Name: $VV_{(Li)}$-iCBG-RFP-GusA (Standard Titer, 0.25 mL)
Catalog Number: OV4009
Lot Number: OV-IM31

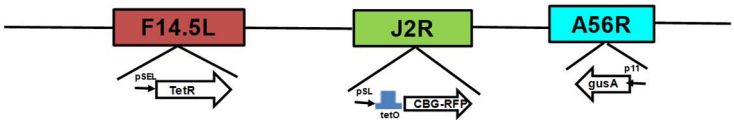
Shipping conditions: Dry ice
Storage conditions: Store at ≤ -70°C upon receipt, avoid subjecting to multiple freeze-thaw cycles

Species: Vaccinia
Virus strain: Lister (Li)
Transgenes: tet-repressor (tetR)
Red fluorescence protein (RFP)
Click beetle green luciferase (CBG)
β-glucuronidase (GusA)

Production Cell Line: A549
Storage Media: 1mM TrisCl, pH 9
Propagation: Inoculate producer cells (e.g. A549) 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.

$VV_{(Li)}$-iCBG-RFP-GusA is a recombinant vaccinia virus (VV) encoding the following additional transcription units: 1) the tet-repressor (tetR) cDNA under transcriptional control of the VV synthetic early late (pSEL) promoter, 2) the tet-operator sequence *tetO* followed by the 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 the 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. Expression of the CBG-RFP fusion protein is under the control of an inducible promoter-system, whereby addition of doxycycline induces the expression of CBG-RFP fusion protein (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:

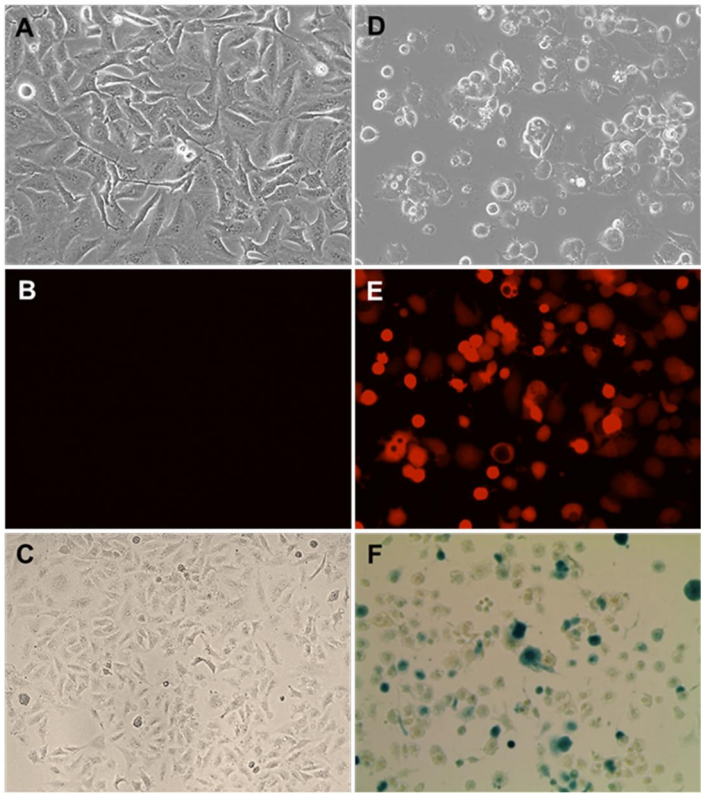
All culture work with Vaccinia should be performed by trained personnel and performed under BSL2 containment following NIH guidelines.

Certificate of Analysis

Testing performed by Imanis Life Sciences

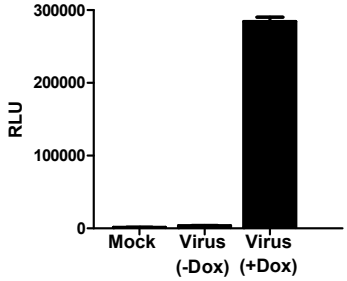
Test description	Result
Titer (on Vero)	2.20 x10^9 $TCID_{50}$ units/mL
Sterility	Pass QC
Cytopathic Effect	Pass QC
Endotoxin Test	Pass QC
Fluorescence Expression	Pass QC
Luciferase Expression	Pass QC
DOX inducibility	Pass QC

Infectivity



Mock-infected A549 cells (A-C). $VV_{(Li)}$-iCBG-RFP-GusA infected A549 cells (MOI 0.1) in the presence of 1µg/ml doxycycline at 48 h.p.i. (D-F). For β-glucuronidase activity, cells were incubated with media containing X-Gluc (5-bromo-4-chloro-3-indolyl-beta-glucuronide) (C&F).

Luciferase Expression



Vero cells were mock-infected or infected with $VV_{(Li)}$-iCBG-RFP-GusA (MOI of 0.1) in the presence (+Dox) or absence (-Dox) of 1µg/ml doxycycline. After 48 hours Click beetle luciferase substrate d-luciferin 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: 15-Feb-2018

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