$VV_{(Li)}$-iTyr-GusA

Product Description

Product Name: $VV_{(Li)}$-iTyr-GusA
(Standard Titer, 0.25 mL)
Catalog Number: OV4011
Lot Number: OV-IM33

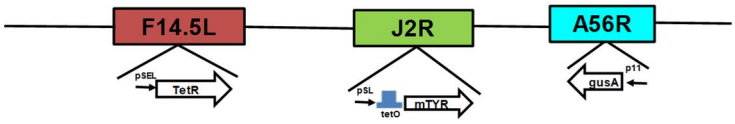
Shipping conditions: Dry ice
Storage conditions: Store at ≤ -70°C upon receipt, avoid subjecting to multiple freeze-thaw cycles

Species: Vaccinia
Virus strain: Lister (Li)
Transgenes: Tet-repressor (tetR)
Mouse Tyrosinase (mTYR)
β-glucuronidase (GusA)

Production Cell Line: A549
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.

$VV_{(Li)}$-iTyr-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 mouse tyrosinase (mTYR) 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. Expression of tyrosinase is under the control of an inducible promoter-system, whereby addition of doxycycline induces tyrosinase expression (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:

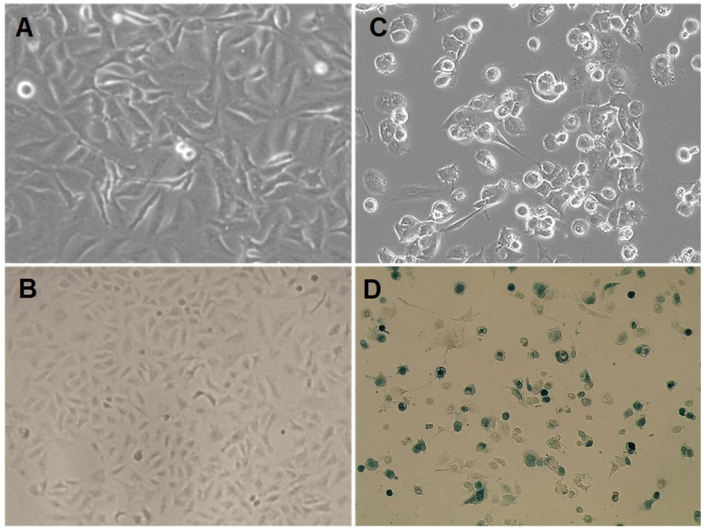
All culture work with Vaccinia should be performed by trained personnel and performed under BSL2 containment following NIH guidelines.

Certificate of Analysis

Testing performed by Imanis Life Sciences

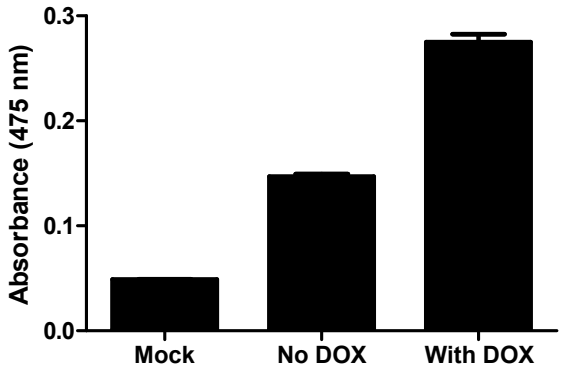
Test description	Result
Titer (on Vero)	1.19×10^9 $TCID_{50}$ units/mL
Sterility	Pass QC
Cytopathic Effect	Pass QC
Endotoxin Test	Pass QC
Tyrosinase Expression	Pass QC
DOX inducibility	Pass QC

Infectivity



A&B: Mock-infected A549 cells
C&D: $VV_{(Li)}$-iTyr-GusA infected A549 cells (MOI 0.1) at 48 h.p.i.
B&D: β-glucuronidase activity: Cells were incubated with media containing X-Gluc (5-bromo-4-chloro-3-indolyl-beta-glucuronide).

Tyrosinase Expression



A549 cells were mock-infected or infected with $VV_{(Li)}$-iTyr-GusA at an MOI of 0.1 in the presence (With DOX) or absence (No DOX) of 1µg/ml doxycycline (DOX). After 48 hours, cell lysates were harvested. To measure tyrosinase expression, 20 µg of cell lysates were incubated at 37ºC for 2h with 50 µL of 2mM L-DOPA. Absorbance was measured using a micro plate reader at 475nm.

Quality Control by: MT
Quality Assurance by: RLV
Effective Date: 15-Feb-2018

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