$VV_{(Li)}$-RlucGFP-mTyr-GusA

Product Description

Product Name: $VV_{(Li)}$-RlucGFP-mTyr-GusA (Standard Titer, 0.25 mL)
Catalog Number: OV4002
Lot Number: OV-IM13

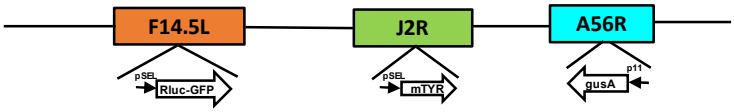
Shipping conditions: Dry ice
Storage conditions: Store at ≤ -70°C upon receipt, avoid subjecting to multiple freeze-thaw cycles

Species: Vaccinia
Virus strain: Lister (Li)
Transgenes: Murine Tyrosinase (mTYR)
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. 48hrs after infection, harvest culture supernatant and cells. Clarify with low-speed centrifugation and resuspend the pellet with 1mM TrisCl (pH 9). Freeze-thaw or sonicate 3 cycles and clarify the supernatant by low-speed centrifugation. Purify through sucrose cushion. Resuspend the viral pellet in 1mM TrisCl, pH 9.

$VV_{(Li)}$-RlucGFP-mTyr-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 murine tyrosinase (mTYR) cDNA under transcriptional control of the pSEL promoter, and 3) the β-glucuronidase (GusA) cDNA under transcriptional control of the VV late P11 (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:

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:

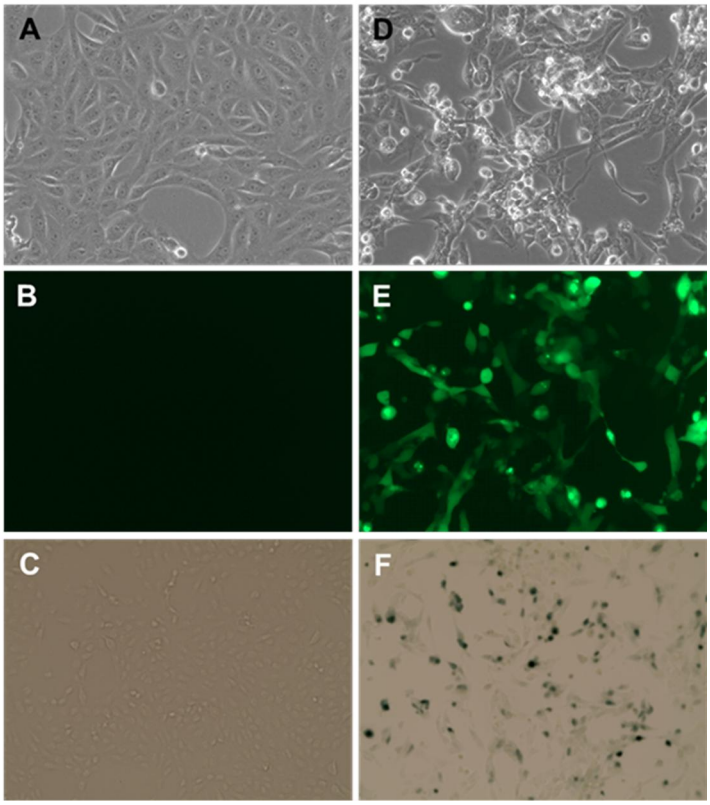
All culture work with Vaccinia should be performed by trained personnel and performed under BSL2 containment following NIH guidelines.

Certificate of Analysis

Testing performed by Imanis Life Sciences

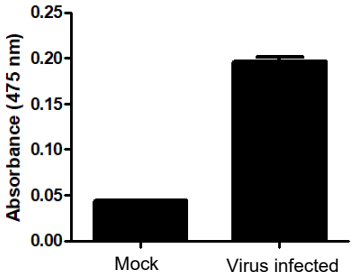
Test description	Result
Titer (on Vero)	5.25 x10^7 $TCID_{50}$ units/mL
Sterility	Pass QC
Cytopathic Effect	Pass QC
Endotoxin Test	Pass QC
Fluorescence Expression	Pass QC
Luciferase Expression	Pass QC
β-glucuronidase Expression	Pass QC
Tyrosinase Expression	Pass QC

Infectivity



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

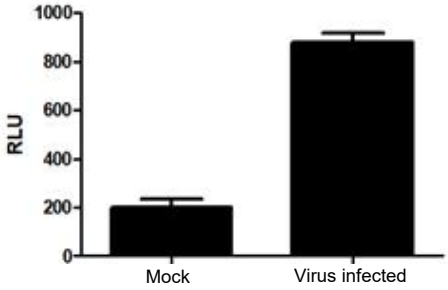
Tyrosinase expression



Vero cells were mock-infected or infected with $VV_{(Li)}$-RlucGFP-mTyr-GusA at an MOI of 0.1. After 48 hours, cell lysates were harvested. To measure tyrosinase expression, 20 µg cell lysates were incubated at 37ºC for 2h with 50 µL of 2mM L-DOPA. Absorbance at 475nmwas measured using a micro plate reader.

$VV_{(Li)}$-RlucGFP-mTyr-GusA

Renilla Luciferase Expression



Vero cells were mock-infected or infected with $VV_{(Li)}$-RlucGFP-mTyr-GusA (MOI=1). After 48 hours, 1.25 µg of coelenterazine 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:16-Nov-2017

Legal Disclaimers

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Imanis Life Sciences. Imanis Life Sciences shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

In no event shall Imanis Life Sciences be liable for any use by Recipient of the Material or for any loss, claim, damage, or liability, of any kind or nature, that may arise from or in connection with the recipient's use, handling, storage, or disposal of the Material, except as such claims, demands, costs, or judgments may arise from Imanis Life Sciences' gross negligence or willful misconduct. Recipient assumes responsibility for, and agrees to indemnify and hold harmless Imanis Life Sciences and Imanis' trustees, officers, agents, and employees from any liability, loss, or damage they may suffer as a result of any claims, demands, costs, or judgments against them arising out of the use, handling, storage, or disposal of the Material by Recipient, except as such claims, demands, costs, or judgments may arise from Imanis Life Sciences' gross negligence or willful misconduct.

For research use only. This certificate is a declaration of analysis at the time of manufacture

PURCHASER NOTIFICATION

LIMITED LICENSE NOTICE – RESEARCH USE ONLY

BY SUBMITTING A SALES ORDER AND OPENING THE PACKAGING OF ORIGINAL MATERIAL, RECIPIENT AGREES TO BE BOUND TO THE TERMS OF THE MATERIAL TRANSFER AGREEMENT ENCLOSED WITH THE PRODUCT. IF RECIPIENT DOES NOT AGREE TO THESE TERMS, DO NOT OPEN THE PACKAGING ON THE ORIGINAL MATERIAL.

1. Imanis Life Sciences has exclusive licenses to sell recombinant measles and Vesicular stomatitis viruses for research use. The sale, license, lease, export, transfer or other distribution of the Material to entity not party to this MTA for financial gain or other commercial purposes and/or the use of the Material to provide a service to a person or entity not party to this MTA for financial gain; or to produce or manufacture for general sale or products for use in the manufacture of products ultimately intended for general sale is STRICTLY PROHIBITED.

2. Limited Use of Material. Recipient may use the Original Material and Progeny only under the direction of Recipient's Scientist for the Research, subject to all of the restrictions set forth herein. No person authorized to use the Material shall be allowed to take or send the Original Material or any Progeny to any location other than the Recipient Scientist's Address, even within Recipient's organization, without Imanis Life Sciences' written consent.

3. Limited Rights Granted. All Material is and remains the property of Imanis. Legal title to the Material shall be unaffected by this Agreement or the transfer made hereunder, and nothing in the Agreement grants Recipient any rights under any patents nor any rights to use the Material or any Modifications or process(es) derived from or with the Material for profit-making or Commercial Purposes. Nothing in this Agreement shall alter any rights the U.S. Government may have with respect to the Material.

4. Not for human or veterinary use. The Material is for research use only and NOT TO BE USED IN HUMANS OR VETERINARY ANIMALS (including for diagnostic purposes). All research involving the Material (including but not limited to research involving the use of animals and recombinant DNA) and disposal of the Material shall be conducted in accordance with all federal, state, local, import/export and other laws, regulations, and ordinances governing such research including applicable NIH guidelines. Recipient will be solely responsible to obtain any necessary governmental permits or approvals to receive and handle the Material.

5. Disclaimers. Any Material delivered pursuant to this Agreement is understood to be experimental in nature, and IMANIS LIFE SCIENCES MAKES NO REPRESENTATIONS AND EXTENDS NO WARRANTIES OF ANY KIND, EITHER EXPRESS OR IMPLIED. THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE MATERIAL WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER RIGHTS.