Quality control report #Doc-000100 | V. 001

#### Lot #LVSA-0100

# LV-Pseudovirus Spike SARS-CoV-2 (B.1.1.7) GFP reporter



The lot number #LVSA-0100 is a lentivirus-based pseudovirus pseutotyped with the SARS-CoV-2 B.1.1.7 (Alpha) variant spike protein. This quality control report demonstrates that the lot #LVSA-0100 is efficient for cell transduction and can be effectively neutralized by a standard neutralizing antibody.

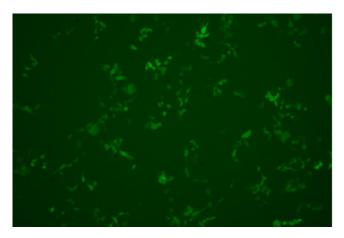
### 1. Transduction efficiency assay

Target cells HEK293 cells (ACE2+, TMPRSS2+)

**Volume of pseudoviruses** 10  $\mu$ L/well

**Detection signal** Fluorescence (GFP)

**Detection method** Fluorescent microscopy



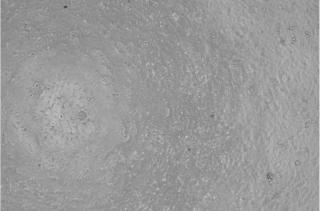


Figure 1: GFP-positive cells after transduction with the LV-Pseudovirus #LVSA-0100.

A volume of 10  $\mu$ l of LV-pseudovirus was mixed to 40  $\mu$ L of culture complete medium, in a 96-well plate. Then, an additional volume of 50  $\mu$ L containing 30 000 HEK293 cells (ACE2+, TMPRSS2+) were seeded in each well. GFP expression analysis was performed 48 hours post-infection by fluorescent microscopy.

Conclusion	The production batch of LV-Pseudoviruses	#LVSA-0100
	can transduce the target cells.	

Quality control report #Doc-000100 | V. 001

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**Lot #LVSA-0100** 

### 2. Neutralization assay

Target cells HEK293 cells (ACE2+, TMPRSS2+)

**Volume of pseudoviruses** 10 μL/well

Neutralizing antibody standard Anti-Spike Protein (RBD) [CV30], AB02019-12.1

**Detection signal** Fluorescence (GFP)

**Detection method** Flow cytometry

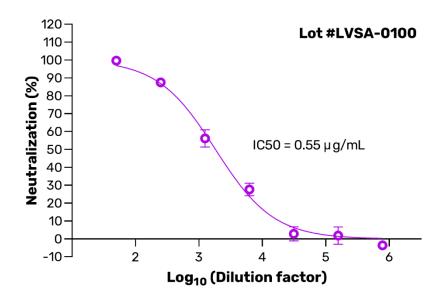


Figure 2: Neutralization curve of the batch #LVSA-0100.

A neutralizing antibody (AB02019-12.1) was serial diluted in a final volume of 50  $\mu$ L of complete medium and incubated for 1 hour with 10  $\mu$ L of LV-pseudoviruses, in a 96-well plate. Then, an additional 50  $\mu$ L containing 30 000 HEK293 cells (ACE2+, TMPRSS2+) were seeded in each well and incubated for 48 hours before flow cytometry analysis. Data in relative unit fluorescence (RFU) were obtained from the analysis of 10 000 cells. Control conditions with pseudoviruses or cells only were also included in the assay as negative controls. The normalized values are analyzed by choosing a nonlinear regression analysis followed by a log (inhibitor) vs response – variable slope function in GraphPad Prism software. Data are representative of duplicates.

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The production batch of LV-Pseudoviruses #LVSA-0100 can be efficiently neutralized by a neutralizing antibody.

Quality control report #Doc-000100 | V. 001

#### Lot #LVSA-0100

**Pseudotyping** 

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### 3. Additional information

Caution We recommend determining the optimal pseudovirus volume to

use according to your specific experimental conditions.

**LV-pseudovirus** 3rd generation, replication incompetent

SARS-CoV-2 B.1.1.7 (Alpha) variant spike protein (GenBank:

MN908947) with multiple mutations including:  $\Delta$ H69/V70,  $\Delta$ Y144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H. The

spike protein has an 18-aa cytoplasmic tail truncation for optimal

infection.

Glycosylation origin Human

**Reporter protein** Green fluorescent protein

**Contact us for more information** <u>mathias.mangion@ivanobioscience.com</u>