IVANO BIOSCIENCE

Pseudovirus Ebola Zaire (GFP)

Lot #PsV-EBZGF-0100

Quality control report

1. Summary

The lot number #PsV-EBZGF-0100 is a lentivirus-based pseudovirus pseutotyped with the glycoprotein of the Ebola Zaire variant. This quality control report demonstrates that the lot #PsV-EBZGF-0100 is efficient for cell transduction and can be effectively neutralized by a standard neutralizing antibody.

2. Transduction efficiency assay

Target cells	HEK293 cells (TIM-1$^+$)
Volume of pseudovirus	0, 1, 5, 10, 20 µL/well
Detection signal	Fluorescence (GFP)
Detection method	Fluorescent microscopy

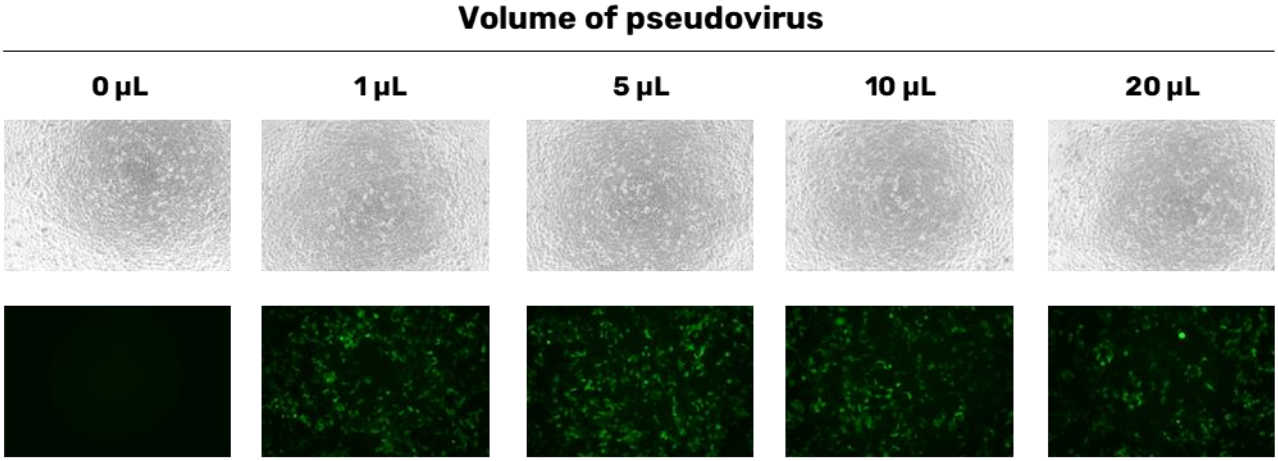

Figure 1: Ebola Zaire pseudovirus transduction efficiency.

A volume range of pseudovirus was mixed in a final volume of 50 µL of culture complete medium, in a 96-well plate. Then, an additional 50 µL containing 20 000 HEK293 cells (TIM-1$^+$) was seeded in each well. GFP expression analysis was performed 48 hours post-infection by fluorescent microscopy. Microscopy parameters: objectif x20, exposure time 200 ms, instrument Leica DMIL LED Fluorescent

Target cells	HEK293 cells (TIM-1$^+$)
Volume of pseudovirus	0, 1, 5, 10 or 20 µL/well
Detection signal	Fluorescence (GFP)
Detection method	Flow cytometry

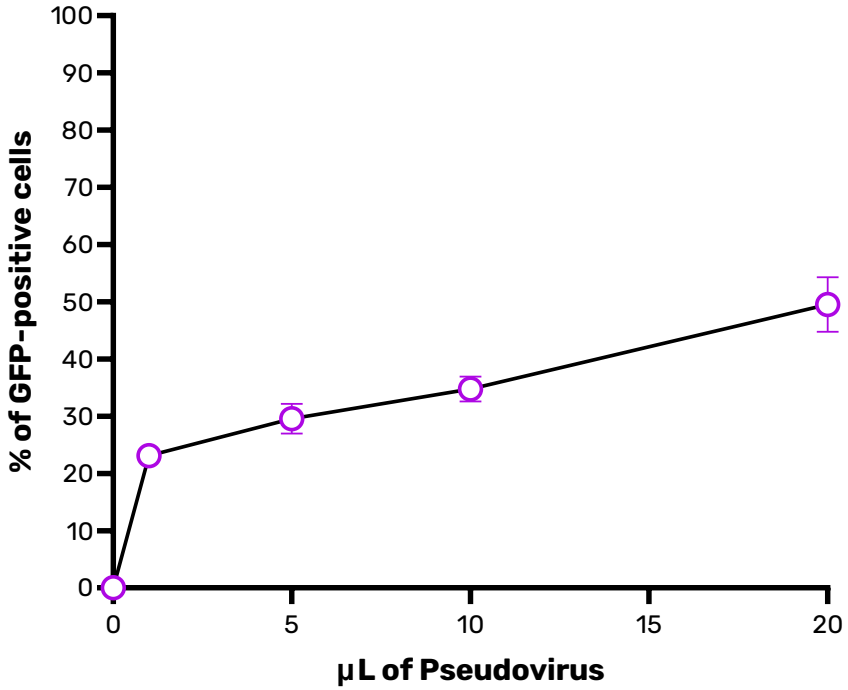

Figure 2: Ebola Zaire pseudovirus transduction efficiency curve.

A volume range of pseudovirus was mixed in a final volume of 50 µL of culture complete medium, in a 96-well plate. Then, an additional 50 µL containing 20 000 HEK293 cells (TIM-1$^+$) was seeded in each well. GFP expression analysis was performed 48 hours post-infection by flow cytometry analysis (at least 10^4 cells).

Conclusion	The GFP Ebola Zaire pseudovirus (#PsV-EBZGF-0100) can transduce the target cells.

3. Neutralization assay

Target cells	HEK293 cells (TIM-1$^+$)
Volume of pseudovirus	5 µL/well
Neutralizing antibody (Nabs)	Anti-Ebola surface glycoprotein[KZ52], AB00690-10.0
Detection signal	Fluorescence (GFP)
Detection method	Fluorescent microscopy

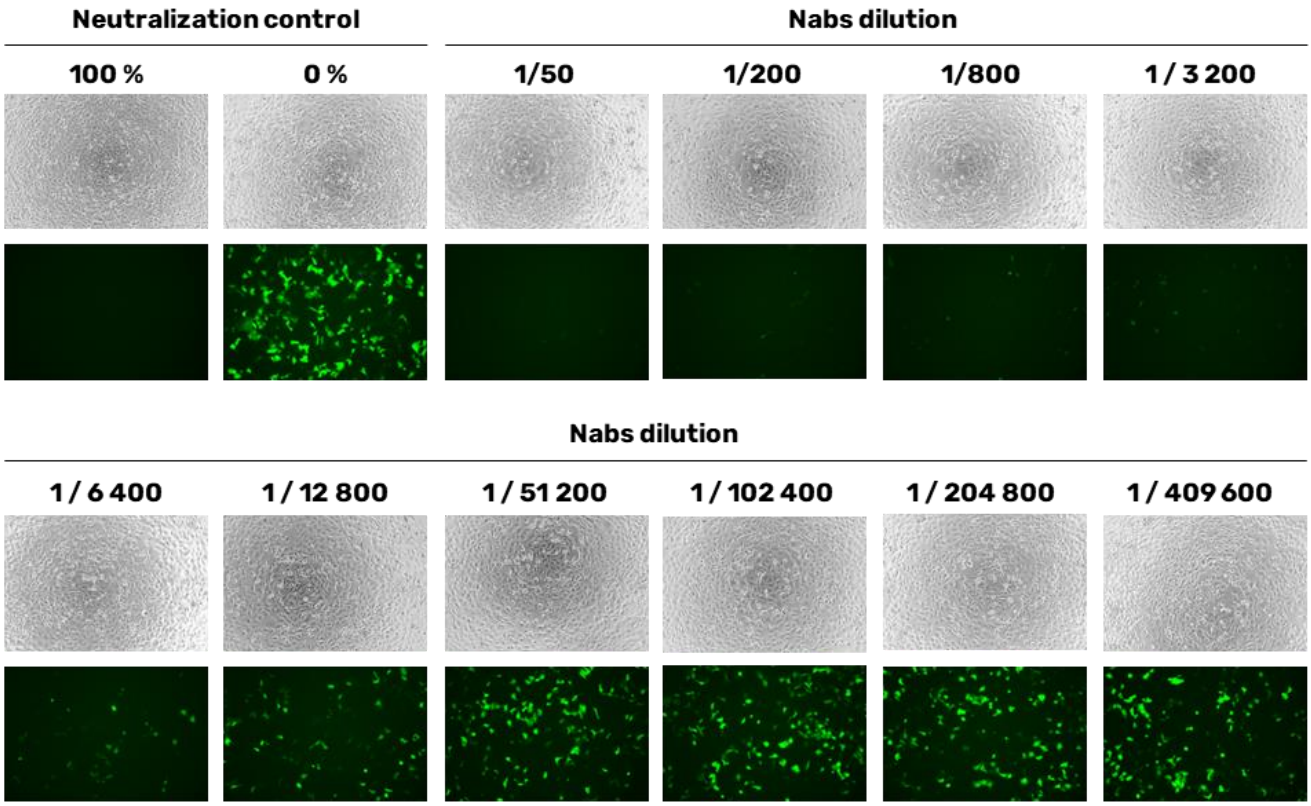

Figure 3: Ebola Zaire pseudovirus neutralization assay.

A monoclonal neutralizing antibody (AB00690-10.0) at 1 µg/µL was serial diluted in a final volume of 50 µL of complete medium and incubated for 1 hour at 37 °C, with 5 µL of pseudovirus, in a 96-well plate. Then, an additional 50 µL containing 20 000 HEK293 cells (TIM-1$^+$) was seeded in each well and incubated for 48 hours before microscopy analysis. Microscopy parameters: objectif x20, exposure time 900 ms, instrument Leica DMIL LED Fluorescent.

Target cells	HEK293 cells (TIM-1$^+$)
Volume of pseudovirus	5 µL/well
Neutralizing antibody (Nabs)	Anti-Ebola surface glycoprotein[KZ52], AB00690-10.0
Detection signal	Fluorescence (GFP)
Detection method	Flow cytometry

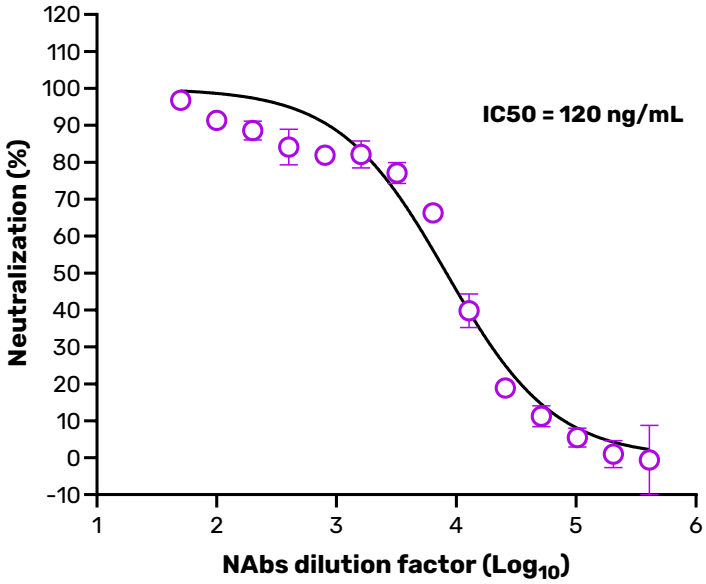

Figure 4 : Neutralization curve.

A monoclonal neutralizing antibody (AB00690-10.0) at 1 µg/µL was serial diluted in a final volume of 50 µL of complete medium and incubated for 1 hour at 37 °C, with 5 µL of pseudovirus, in a 96-well plate. Then, an additional 50 µL containing 20 000 HEK293 cells (TIM-1$^+$) was seeded in each well and incubated for 48 hours before flow cytometry analysis (at least 10^4 cells). Raw data in percentage of GFP-positive cells were analyzed using a log(inhibitor) *vs* normalized-response (variable slope) non-linear regression model in Prism v10 (GraphPad). Percentages of neutralization were normalized considering only cells into wells as 100% neutralization and cells transduced by pseudoviruses without any NAbs as 0% neutralization. Data are representative of duplicates.

Conclusion	The GFP Ebola Zaire pseudovirus #PsV-EBZGF-0100 can be efficiently neutralized by neutralizing antibodies.

3. Additional information

Caution	We recommend determining the optimal pseudovirus volume to use according to your specific experimental conditions.
Pseudovirus	3rd generation, replication incompetent
Pseudotyping	Ebola virus glycoprotein from the 2014 outbreak in west Africa (GENBANK : KP096420.1)
Glycosylation origin	Human
Reporter protein	Green fluorescent protein
Storage	- 80 °C, avoid freeze/thaw cycles
For more information	mathias.mangion@ivanobioscience.com Message object should contain : "#PsV-EBZGF-0100"