



# **Ebola Pseudovirus Zaire Variant**

**Luciferase reporter**

**Lot #251215-EBZ**



## **Certificate of Analysis**

# 1. Summary

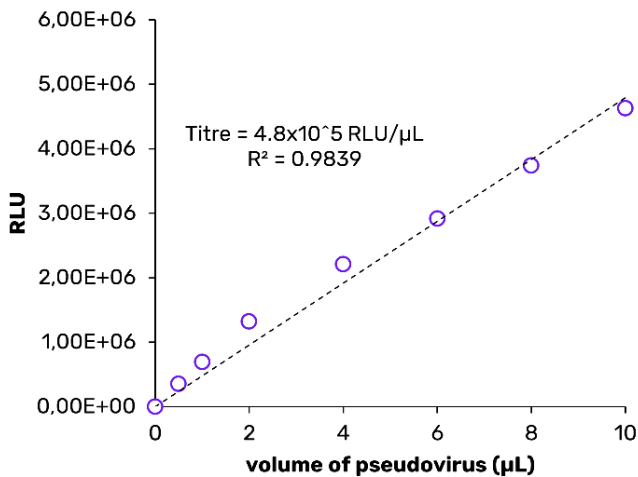
This certificate provides a functional validation of the Ebola pseudovirus, Zaire variant, lot #251215-EBZ. The titer is  $4.8 \times 10^5$  RLU/ $\mu$ L. A 1,000-fold-to-background ratio is obtained with 1  $\mu$ L per well (96-well plate). Hence, a volume of 1 mL is sufficient to perform approximately 1,000 reactions, or  $10 \times 96$ -well plates. According to IVANO Bioscience’s protocol, available upon request.

# 2. Transduction efficiency assay

**Target cells** HEK293T cells  
**Volume of pseudovirus** 0 – 0.5 – 1 – 2 – 4 – 6 – 8 – 10  $\mu$ L/well  
**Detection signal** Luminescence (firefly luciferase)  
**Detection method** Microplate reader Biotek Synergy H1 (Gain: 135)

### Titration Curve

(PSVEBZ - Lot #251215-EBZ)



Volume of pseudovirus ( $\mu$ L)	RLU 1	RLU 2	Mean	CV	Fold to Background
0	3,6E+02	3,0E+02	3,3E+02	4,3E+01	1,0E+00
0,5	3,6E+05	3,5E+05	3,6E+05	5,9E+03	1,1E+03
1	6,6E+05	7,3E+05	6,9E+05	5,3E+04	2,1E+03
2	1,3E+06	1,3E+06	1,3E+06	4,3E+03	4,0E+03
4	2,2E+06	2,2E+06	2,2E+06	2,5E+04	6,6E+03
6	2,9E+06	3,0E+06	2,9E+06	8,7E+04	8,8E+03
8	3,8E+06	3,7E+06	3,7E+06	5,5E+04	1,1E+04
10	4,6E+06	4,6E+06	4,6E+06	1,0E+04	1,4E+04

### Figure 1: Transduction efficiency curve

A volume range of pseudovirus was mixed in a final volume of 50  $\mu$ L of medium, in a 96-well plate. Then, 50  $\mu$ L of medium containing 10,000 cells was seeded in each well. On the day of analysis, an additional 100  $\mu$ L of Bright-Glo Luciferase reagent was added in each well and incubated for 2 minutes. Data in relative unit luminescence (RLU) were obtained from the analysis of 150  $\mu$ L of the cell lysate, using a microplate reader. Data are expressed in relative unit luminescence (RLU).

### Conclusion

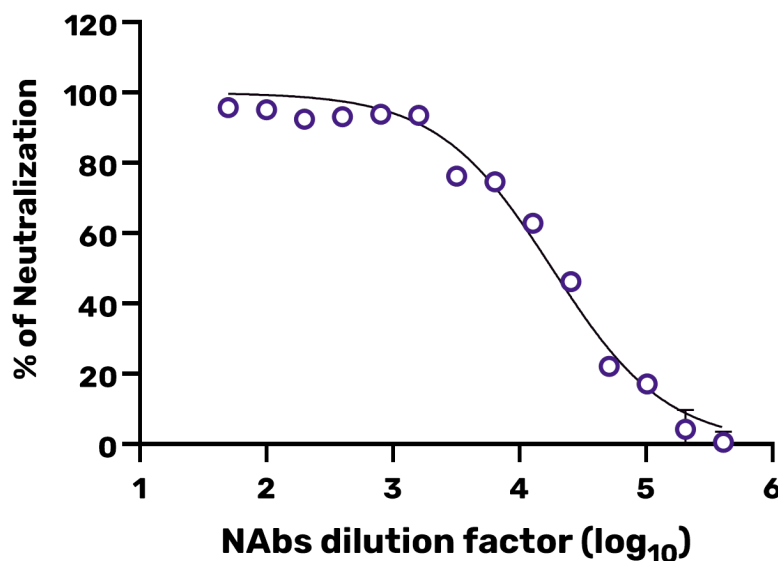
The Ebola pseudovirus, Zaire variant (lot #251215-EBZ), is capable of transducing target cells. The titer is  $4.8 \times 10^5$  RLU/ $\mu$ L. Using 1 microliters of pseudovirus per reaction in a 96-well plate results in a 1,000-fold increase in RLU compared to the background. Accordingly, 1 mL of lot #251215-EBZ can be used to perform approximately 1,000 reactions, or  $10 \times 96$ -well plates, according to IVANO Bioscience’s protocol (available upon request)

### 3. Neutralization assay

<b>Target cells</b>	HEK293T cells
<b>Volume of pseudovirus</b>	1 $\mu$ L/well
<b>Neutralizing antibody (Nabs)</b>	Anti-Ebola surface glycoprotein [KZ52] , Ab00690-10.0
<b>Detection signal</b>	Luminescence (firefly luciferase)
<b>Detection method</b>	Microplate reader Biotek Synergy H1 (Gain: 135)

#### Neutralization curve

(PSVEBZ - lot #251215)



**Figure 2: Neutralization curve**

A monoclonal neutralizing antibody (Ab00690-10.0), at a starting dilution of 1/50, was serially diluted in a final volume of 50  $\mu$ L of complete medium and incubated for 1 hour at 37  $^{\circ}$ C, with 1  $\mu$ L of pseudovirus, in a 96-well plate. Then, an additional 50  $\mu$ L containing 10,000 cells was seeded in each well and incubated for 72 hours. Finally, an additional 100  $\mu$ L of Bright-Glo™ Luciferase buffer was added in each well and incubated for 2 minutes. Data in relative unit luminescence (RLU) were obtained from the analysis of 150  $\mu$ L of the cell lysate with a microplate reader. Raw data 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 in wells as 100% neutralization and cells transduced by pseudoviruses without any NAbs as 0% neutralization. Data are representative of duplicates. ).

#### Conclusion

The Ebola pseudovirus, Zaire variant (lot #251215-EBZ) can be efficiently neutralized by neutralizing antibodies.

## 4. Additional information

<b>Intruction of use</b>	We recommend determining the titer in your lab's conditions before performing any experiments Handle under biosafety level-2
<b>Pseudovirus</b>	3 <sup>rd</sup> generation lentiviral vector, incompetent replication and non-toxic
<b>Pseudotyping</b>	Glycoprotein of the Ebola pseudovirus, Zaire variant Genbank: AIW47454.1
<b>Pseudotyping sequence</b>	MGVTGILQLPRDRFKRTSFFLWVILFQRTFSIPLGVIHNSTLQVSDVDKLVCRDK LSSTNQLRSVGLNLEGNVATDVPSVTKRWGFRSGVPPKVVNYEAGEWAENC YNLEIKKPDGSECLPAAPDGIRGFPRCRYVHKVSGTGPCAGDFAFHKEGAFFLY DRLASTVIYRGTTFAEGVVAFLILPQAKKDFSSHPLRELVNATEDPSSGYSTTI RYQATGFGTNETEYLFVVDNSTYVQLESRFTPQFLLQLNETIYASGKRSNTTGK LIWKVNPEIDTTIGEWAFRETKKNLTKIRSEELSFTAVSNGPKNISGQSPARTSS DPETNTTNEDEHKIMASENSSAMVQVHSQGRKAAVSHLTTLATISTSPQPPTTKT GPDNSTHNTVPYKLDISEATQVGQHHRRADNDSTASDTPPATAAGPLKAENT NTSKSADSLDLATTTSPQNYSETAGNNNTHHQDTGEESASSGKLGITNTIAGV AGLITGRRTRREVIVNAQPKCNPNLHYWTTQDEGAAIGLAWIPYFGPAAEGIYI EGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSILNRKAIDFLLQRWGG TCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDKTLPDQGDNDNWWTGWRQWIP AGIGVTGVIIAVALFCICKFVF
<b>Glycosylation origin</b>	Human
<b>Reporter Protein</b>	Firefly luciferase
<b>Storage</b>	- 80 °C, avoid freeze/thaw cycles
<b>For more information</b>	mathias.mangion@ivanobioscience.com Message object should contain: "PSVEBZ – lot #251215-EBZ "
<b>Intended use</b>	For Research Use Only Not for Use in Diagnostic Procedures, not Meant for Resale