PSEUDOVIRUS

products@ibtbioservices.com

Influenza H5 Pseudovirus

Catalog #: 1005-PP-005

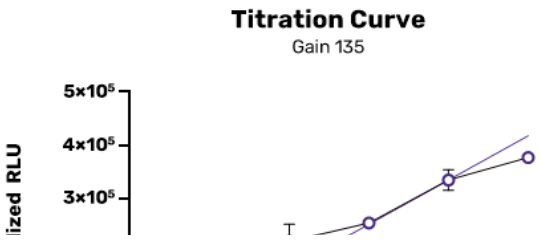
Description:
The influenza H5 pseudovirus is a third-generation lentiviral vector pseudotyped with the hemagglutinin protein from the influenza A/VietNam/1194/2004 (H5N1) strain. It contains a firefly luciferase reporter system. This pseudovirus is classified under biosafety level 2 (BSL-2) and is not known or suspected to contain any replicative biological agents due to multiple safety modifications in the viral genome (e.g.: altered 3' LTR rendering the vector self-inactivating, exclusion of the viral tat gene, and replacement of the U3 region in the 5' LTR with a strong tat-independent constitutive promoter).

Formulation & Storage: Supplied in DMEM supplemented with 10% Fetal Bovine Serum.
Store at -80°C. Avoid Thaw/freeze cycle.

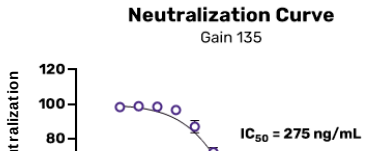
Notes & Usage Guidelines:
Recommended Dilution: 1:50 to 1:100
Luciferase Units for Assays: 40 000 to 80 000
Reporter: Luciferase
~No. of plates that can be tested with a single aliquot: 1 plate

Applications

Viral Titration:
Pseudoviruses were serially diluted 2-fold in DMEM supplemented with 2% FBS, 25 mM HEPES, and 1x GlutaMAX (transduction medium). Next, 50 µL of transduction medium containing 10,000 HEK293-T cells was added to each well. Twenty-four hours post-transduction, 100 µL of DMEM supplemented with 10% FBS, 25 mM HEPES, and 1x GlutaMAX was added. Luciferase expression was measured 72 hours post-transduction using the Bright-Glo™ Luciferase reagent (Promega, Madison, WI, USA) according to the manufacturer's instructions. Analysis was performed using a Biotek Synergy H1 plate reader (Gain: 135). Cells with media alone were used as a control. Data are representative of duplicates. Viral titer was expressed as Relative Luciferase Units per microliter (RLU/µL). The titration obtained may vary depending on the user's instrument, handling, or luciferase assay system.



Pseudovirus Neutralization Assay Protocol:
A monoclonal neutralizing antibody (NAb) (Absolute Antibody, cat# AB00798-10.0) at 20 µg/mL was serially diluted 2-fold in a final volume of 50 µL of transduction medium and incubated for 1 hour at 37 °C with 1 µL of pseudovirus in a 96-well plate. Next, 50 µL of transduction medium containing 10,000 HEK293-T cells was added to each well. Twenty-four hours after pseudovirus addition, 100 µL of DMEM supplemented with 10% FBS, 25 mM HEPES, and 1x GlutaMAX was added. Luciferase expression was measured 72 hours post-transduction using the Bright-Glo™ Luciferase reagent (Promega, Madison, WI, USA) according to the manufacturer's instructions. Analysis was performed on a Biotek Synergy H1 plate reader (Gain: 135). Raw data were normalized by considering cells in wells with no pseudovirus as 100% neutralization and cells transduced by pseudovirus without NAbs as 0% neutralization. The normalized data were then analyzed using a log(inhibitor) vs normalized response (variable slope) non-linear regression model (GraphPad Prism). Data are representative of duplicates.



Certificate of analysis:
A hardcopy of datasheet sent along with the products. Please refer to it for detailed information. For older lots, refer to the applicable certificate of analysis that may be requested at services@ibtbioservices.com

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