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Antibody Datasheet

Product Name: Mouse anti Ebola Virus VP40 protein (Zaire/Sudan)

Clone number: 5B5

Isotype: Mouse IgG_{2b}

Product code: MAB12169-100

Batch Number:

Amount: 0.1mg

Concentration: 1 mg/ml

Buffer: Phosphate Buffered Saline pH7.2

Preservative: 0.09% Sodium Azide (NaN_3)

Purification: The antibody was purified by affinity chromatography on protein A

Immunogen: Recombinant Ebola virus VP40 protein

Specificity: This antibody is specific for viral matrix protein (VP40) of Zaire and Sudan Ebola virus species.

Applications: ELISA, WB

Antigen background: Ebola virus disease (EVD) is a severe disease caused by several species of *Ebolavirus* (EBOV), of the family *Filoviridae*. *Ebolavirus* are enveloped, negative-sense, single-stranded, RNA viruses. Prior to 2007, four species of *Ebolavirus* were recognised including *Zaire, Sudan, Reston* and *Tai Forest*. The presence of a fifth EBOV virus species, *Bundibugyo ebolavirus* (BEBOV) was identified after an outbreak of EVD in the Bundibugyo District of western Uganda in 2007.

Three of these virus species, *Zaire, Sudan* and *Bundibugyo* ebolavirus have caused significant disease outbreaks in humans. The *Zaire* subtype of the Ebola virus family is currently the most important in relation to outbreaks of disease in humans. The *Zaire* subtype was responsible for the largest ever outbreak of EVD, which started in West Africa in 2014.

Studies suggest that fruit bats of the *Pteropodidae* family act as natural hosts for the Ebola virus. Transmission of EBOV to humans is thought to occur through direct contact with sick or dead wild animals that have been infected by the virus. Outbreaks of EVD are associated with person-to-person transmission after the virus is introduced into humans from the zoonotic reservoir (WHO).

During outbreaks the virus is commonly transmitted through direct contact with infected persons or their bodily fluids. The onset of EVD is associated with nonspecific clinical symptoms, including fever, myalgia, headache, abdominal pain, nausea, vomiting, and diarrhoea. Common symptoms of EVD also include fever with a rash appearing around the face, trunk and arms. In the later stages of disease, overt haemorrhage can occur and has been reported in up to 50% of cases. Severe cases of EVD can be fatal (MacNeill, A).

The severity of disease varies according to the species of EBOV involved, but the molecular mechanism influencing virulence and pathogenesis has not been fully elucidated. However, studies suggest that EBOV glycoprotein (GP) may be responsible for critical pathogenic differences among EBOV species.

References:

World Health Organization: Ebola virus disease

Adam MacNeil et al (2010). Proportion of Deaths and Clinical Features in Bundibugyo Ebola Virus Infection, Uganda. Emerg Infect Dis. 16(12): 1969–1972.

Storage:

Store at +4^{0}C for up to three months, or at -20^{0}C for longer.
The Antibody is shipped at ambient temperature.
Avoid repeated freeze/thaw cycles.

Indirect ELISA

Coating: Maxisorp ELISA plates were coated with 200ng, 20ng or 2ng of ZEBOV VLP (virus-like particles) or no antigen in 200mM sodium carbonate pH9.2 overnight at 4°C.

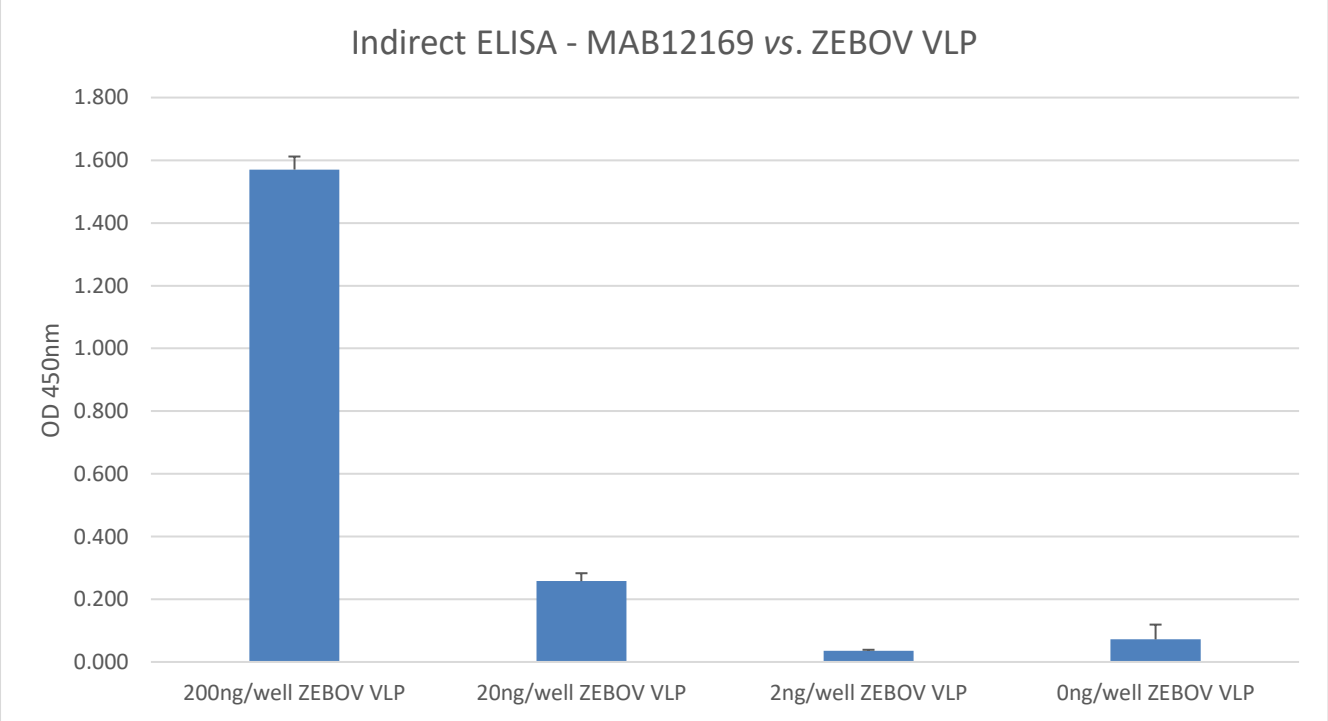
Blocking: 2% BSA in 200mM sodium carbonate pH9.2 for 2h at room temperature.

Primary antibody: Mouse ascites 1:2000 dilution in Blocking Buffer (2% BSA in phosphate buffered saline + 0.1% Tween-20) for 1.5h at room temperature.

Secondary antibody: 1:4000 dilution of goat anti-mouse-IgG-HRP (AbDSerotec 70415) in Blocking Buffer (2% BSA in phosphate buffered saline + 0.1% Tween-20) for 1h at room temperature

Development: 1.5 minutes using TMB Surewell substrate (KPL). 1M HCl was used as stop solution.

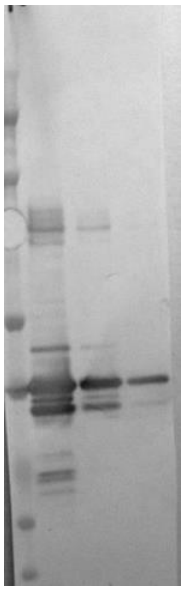
Analysis: Absorption was read at 450nm.



Western Blot

Either 500ng, 100ng or 20ng of ZEBOV VLP (virus-like particles at 0.2mg/ml) were loaded into lanes 2, 3 or 4, respectively. Proteins were resolved on a 4-12% Bis-Tris gradient acrylamide gel (Invitrogen) for 55 minutes at 200V in 1xMOPS running buffer (Invitrogen). Western blots were performed using Transblot and pre-packed nitrocellulose membrane blotting packs (both Biorad) at 12V and 2.5A for 10 minutes. Membranes were blocked in 5% milk powder in PBS-T for 1h at room temperature, then incubated in 10ml of a 1:1000 antibody dilution in Blocking Buffer overnight. Blots were washed 3x in PBS-T, then incubated for 1h with a 1:2000 dilution of goat anti-mouse-IgG-HRP conjugate (Sigma) in Blocking Buffer. Blots were washed 3x in PBS-T, and signal was detected using TMB Membrane substrate (KPL) for ~1 minute.

1 2 3 4



Lane 1: MW marker

Lane 2: 500ng ZEBOV VLP (0.2mg/ml)

Lane 3: 100ng ZEBOV VLP (0.2mg/ml)

Lane 4: 20ng ZEBOV VLP (0.2mg/ml)