

Building B, Langford Locks Kidlington Oxford Oxfordshire , OX5 1LH United Kingdom Tel: +44 (0)1865 595230

## **Antibody Datasheet / Certificate of Analysis**

**Product Name:** Mouse anti-Chikungunya virus Capsid protein

Clone number: 19B02

**Isotype:** Mouse IgG1

**Product code:** MAB12131-200

**Batch Number:** TBC

Immunogen: Inactivated native Chikungunya virus, strain 181/25

Amount: 200ug

**Concentration:** 1mg/ml

**Buffer:** Phosphate Buffered Saline pH7.4

**Preservative:** None present

**Purification:** Antibody was purified by affinity chromatography on Protein A

**Specificity:** This antibody is specific for the Capsid protein of Chikungunya virus.

It demonstrates negligible cross-reactivity with other members of the alphavirus family, including Western Eastern and Venezuelan Encephalitis viruses. There is

no cross-reactivity to Zika, Dengue or other flavivirus antigens.





**Applications:** ELISA, WB, Immunofluorescence

Antigen background: Chikungunya virus is the aetiological agent of chikungunya fever. CHIKV belongs to the Alphavirus genus, and is an enveloped, single-stranded positive-sense RNA virus (Strauss & Strauss, 1994). The alphavirus genome encodes four nonstructural proteins (nsP1 to nsP4) and five structural proteins (capsid, E3, E2, 6K and E1).

> CHIKV is transmitted to humans by *Aedes* mosquitoes, and disease is characterized by a rapid onset of fever, myalgia and often a rash (usually maculopapular), with chronic disease characterized by episodic, and often debilitating, polyarthralgia/polyarthritis. (Suhrbier et al., 2012). The largest epidemic of CHIKV disease ever reported began in 2004 and has since been responsible for up to 6.5 million human cases, primarily in Africa and Asia, with imported cases reported in over 40 countries. CHIKV infection is symptomatically similar to infection with Zika virus and Dengue virus, and differential diagnosis using immunoassay based testing is important in patient management.

> The alphavirus capsid protein (CP) is a multifunctional protein that has been shown to act as a serine protease for self-cleavage, binds viral genomic RNA and other CP molecules during nucleocapsid formation, and interact with viral spike proteins during virion formation and egress (<u>Choi et al., 1991</u>). The CP of CHIKV forms two major domains. The N-terminal domain is implicated in non-specific RNA binding, while the C-terminal domain harbours the globular protease and the binding site for the spike protein ( Hong et al., 2006).

Storage:

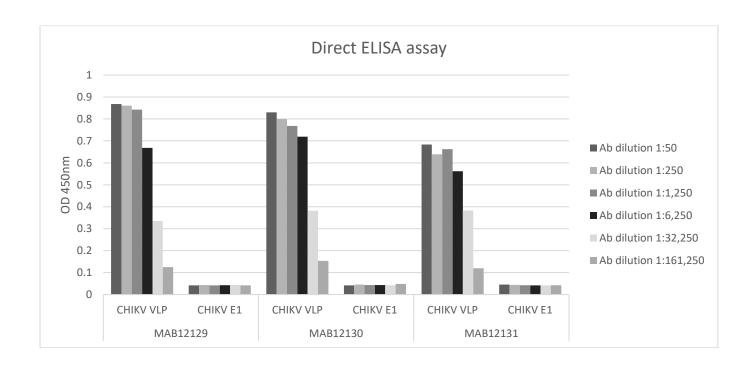
Store at +4°C for up to three months, or at -20°C for longer periods The antibody is shipped at ambient temperature. Avoid repeated freeze/thaw cycles.



## **ELISA**

**Plate preparation:** NUNC Maxisorp plate coated with  $100\mu$ l of  $1\mu$ g/ml CHIKV E1 antigen and CHIKV VLP antigen using Carbonate buffer (pH- 9.47). Incubate the plate at RT for 1 hour. Wash the plate with TBS-T buffer (3X). Add  $300\mu$ l of 5%BSA in 1X DPBS, incubate the plate at RT for 1 hour.

Assay procedure: Prepare 5-fold serial dilutions of antibodies (1:50 to 161,250) using 1% BSA/1XDPBS/0.05%T20 as diluent. Add  $50\mu$ l of the prepared dilutions in the wells, seal the plate and incubate at RT for 1 hour on a rotatory shaker (~500 RPM). Wash the plate with TBS-T wash buffer (3X). Add  $100\mu$ l of goat Anti-mouse IgG-HRP (1:10K) in all the wells and incubate the plate at RT for an hour, on a rotatory shaker (~500 RPM). Diluent used - 1%BSA/1XDPBS/0.05%Tween20. Wash the plate with TBS-T wash buffer (6X). Add  $100\mu$ l of HK-TMB substrate in all the wells and incubate the plate at RT, until colour develops. Add  $50\mu$ l of 1M HCl in all the wells and read the plate at 450nm.







## **Western Blot**

100ng of CHIKV VLP loaded and Western blot performed with 1 $\mu$ g/ml primary antibody overnight at 4°C.

