

Antibody Datasheet

Product Name:	Mouse anti-Zika virus VLP antibody
Clone number:	ID5-2-H7-G3
Isotype:	IgG1 Kappa
Product code:	MAB12312-100 MAB12312-500
Batch Number:	M-NAC-9C
Immunogen:	Recombinant Zika virus VLPs (comprising envelope, pre-membrane and membrane proteins), produced in HEK293 cells and available from the Native Antigen Company here .
Amount:	100µg 500µg
Concentration:	1.0 mg/ml
Buffer:	Phosphate Buffered Saline pH7.4
Preservative:	None present. 0.2µm filtered.
Fusion partners:	Spleen cells from immunised Balb/c mice were fused with cells from the SP2/0-Ag14 myeloma cell line.
Purification:	Antibody was purified from hybridoma cell culture supernatant by affinity chromatography on Protein G.
Specificity:	In ELISA antibody is specific for Zika virus (ZIKV) virus-like particles (VLP) and envelope protein (ENV), and shows no cross-reactivity with envelope proteins from Dengue virus (DENV), Chikungunya virus (CHIKV), West Nile virus (WNV) and Japanese Encephalitis virus (JEV). See data below. In non-reducing Western blot antibody reacts with ZIKV, DENV, JEV and WNV. Antigens were not detected in reduced conditions. See data below.

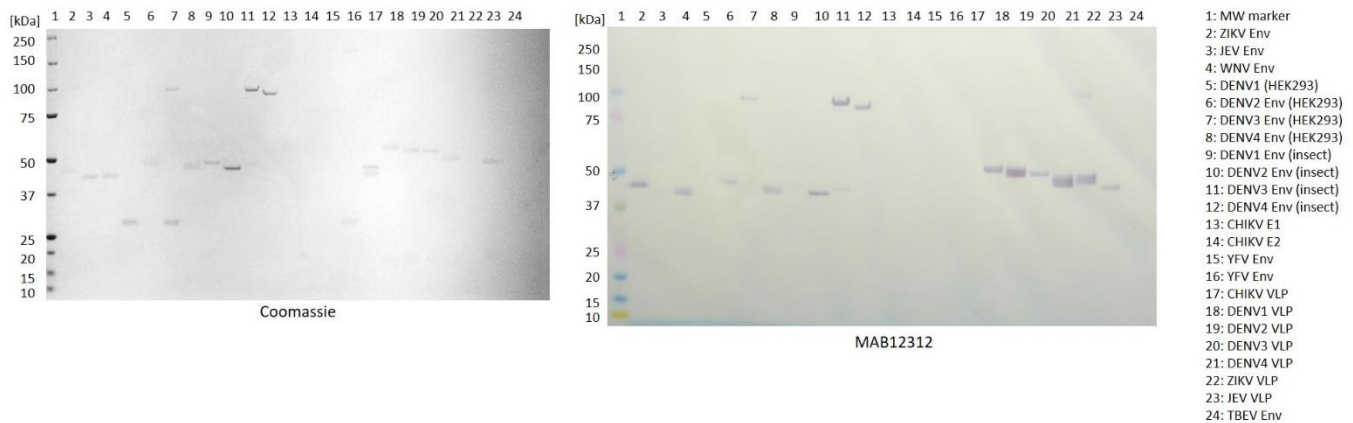
Applications: WB (non-reduced), ELISA

Storage: Store at +4°C for up to one week, or at -20°C for longer periods
For long term storage at +4°C the addition of 0.09% w/v sodium azide is recommended.
The antibody is shipped at ambient temperature.
Avoid repeated freeze/thaw cycles.

Western Blot (non-reduced)

100ng of each antigen was used for SDS-PAGE, in non-reduced form. Proteins were transferred using Transblot for 7 minutes at 25V. 5% dry milk in PBS-T was used as blocking buffer and dilution buffer for antibodies. Primary antibodies are given below, and goat anti-mouse-IgG-HRP secondary antibody (Biorad 103005) was used at 1:1000. All steps were carried out for 1h at room temperature with gentle rocking. KPL Membrane TMB was used for detection. Development time 30 seconds.

Lanes are (1) MW marker, (2) ZIKV Env, (3) JEV Env, (4) WNV Env, (5) DENV1 (HEK293), (6) DENV2 Env (HEK293), (7) DENV3 Env (HEK293), (8) DENV4 Env (HEK293), (9) DENV1 Env (insect), (10) DENV2 Env (insect), (11) DENV3 Env (insect), (12) DENV4 Env (insect), (13) CHIKV E1, (14) CHIKV E2, (15) YFV Env, (16) YFV Env, (17) CHIKV VLP, (18) DENV1 VLP, (19) DENV2 VLP (20) DENV3 VLP, (21) DENV4 VLP, (22) ZIKV VLP, (23) JEV VLP, (24) TBEV Env.



ELISA

- **Plate coating:** All antigens coated at 0.5µg/ml at RT in DPBS for an hour.
- **Plate blocking:** Plate washed 1 X 300µl/well TBS + 0.1% Tween20, blocked 300µl/well DPBS+1% BSA overnight at 2-8C. Plate washed 3 X 300ul/well TBS-T wash buffer.
- **Detection antibody:** Antibody diluted to 1.0µg/ml and 0.01µg/ml in DPBS + 1% BSA + 0.05% T20. Added at 100ul/well, incubated shaken 2h room temperature. Plate washed 3 X 300µl/well TBS-T wash buffer.
- **Secondary antibody:** Biorad goat anti-mouse IgG-HRP (103005) diluted 1 in 2500 in DPBS/1%BSA/0.05%T20, added at 100µl/well, incubated shaken 1h room temperature. Plate washed 6X 300µl/well TBS-T wash buffer.
- **Detection:** Europa TMB substrate added at 100µl/well and the plate developed for 5 min. static on the bench.
- **Stop:** Reaction stopped with 100µl/well 1M HCL and the plate was read within 5 min. at 405nm.

