

The Native Antigen Company LGC Unit 3 Oxford Technology Park Kidlington Oxford Oxfordshire OX5 1GN

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Antibody Datasheet

Name: Mouse anti ZIKA VLP antibody (IG3)

Product Code: MAB12311-100 / MAB12311-500

Batch #: 19050110

Date of Manufacture: 01-MAY-2019

Product Description: Mouse monoclonal antibody specific for Zika virus VLP (clone IG3).

Clone Number: IG3-1-A12-G9

Isotype: IgG

Amount: 0.1 mg / 0.5 mg

Concentration: 1.0 mg/ml

Purity: >95%

Presentation: Liquid

Buffer: PBS pH7.4

Preservative: None present. 0.2μ m filtered.

Immunogen: Recombinant Zika virus VLPs (comprising envelope, pre-membrane and membrane proteins), produced in HEK293 cells and available from the Native Antigen Company here.

Purification: Antibody was purified from hybridoma cell culture supernatant by affinity chromatography on Protein G

Specificity: This antibody recognises Zika virus VLPs and envelope protein. In ELISA it also shows cross-reactivity with VLPs and envelope proteins from Dengue virus (DENV), West Nile virus, Japanese Encephalitis virus and Chikungunya virus. It recognises recombinant His-tagged DENV envelope proteins produced in both mammalian and insect cells. Fc-tagged envelope proteins may not be detected in ELISA. See data below. In non-reduced Western blot antibody detects ZIKV Env and VLP. Antigens were not detected in reduced conditions. See data below.

Applications: WB (non-reduced), ELISA







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Usage Guidelines

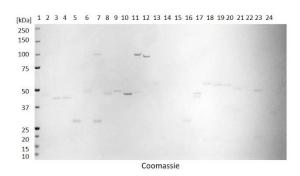
Short Term Storage: +4°C

Long Term Storage: -20°C

Storage Guidelines: The antibody is shipped at ambient temperature. Avoid repeated freeze/thaw cycles. Addition of 0.09% w/v sodium azide is recommended for long-term storage at +4°C.

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 antimouse-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.





1: MW marker 2: ZIKV Env 3: JEV Env 4. WNV Fnv 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: assay was carried out as descried below;

<u>Plate coating:</u> All antigens (virus-like particles, VLP) coated at 0.5μg/ml at RT in DPBS for an hou <u>Plate blocking:</u> 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.

<u>Detection antibody:</u> Antibody diluted to $1.0\mu g/ml$ and $0.01\mu gml$ in DPBS + 1% BSA + 0.05% T20. Added at 100ul/well, incubated shaken 2h room temperature. Plate washed 3 X $300\mu l/well$ TBS-T wash buffer.





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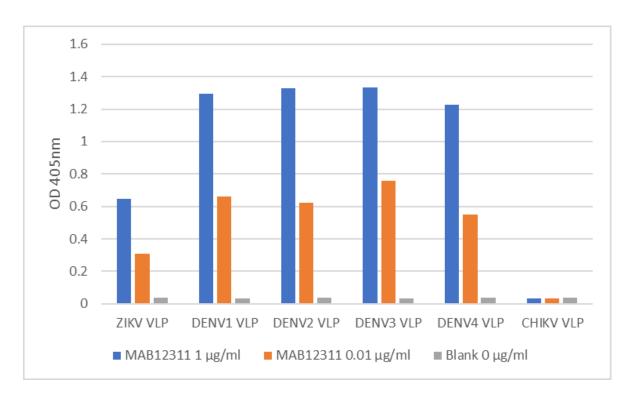
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Secondary antibody: Biorad goat anti-mouse IgG-HRP (103005) diluted 1 in 2500 in DPBS/1%BSA/0.05%T20, added at 100µl/well, incubated shaken 1h room temperature. Plate washed 6X 300µl/well TBS-T wash buffer.

<u>Detection:</u> 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.



ELISA: assay was carried out as descried below;

<u>Plate coating:</u> All antigens (envelope proteins, ENV) coated at $0.5\mu g/ml$ at RT in DPBS for an hour. <u>Plate blocking:</u> Plate washed 1 X $300\mu l/well$ TBS + 0.1% Tween20, blocked $300\mu l/well$ DPBS+1% BSA for 1h. Plate washed 3 X 300u l/well TBS-T wash buffer.

Detection antibody: Antibody diluted to 1.0μg/ml and 0.01μgml in DPBS + 1% BSA + 0.05% T20. Added at 100μl/well, incubated shaken 1h 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 100μ l/well, incubated shaken 1h room temperature. Plate washed 6X 300μ l/well TBS-T wash buffer.

<u>Detection:</u> Europa TMB substrate added at 100µl/well and the plate developed for 5 min. static on the bench.

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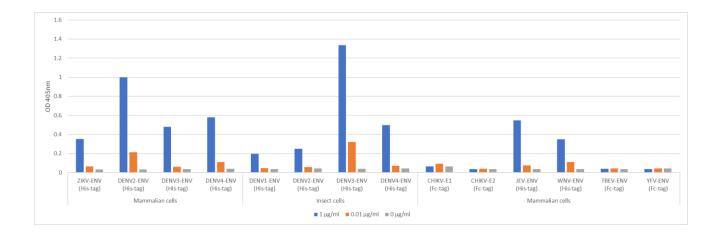
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03/04/2025



QC

Signed by: Tracy.Pullen@lgcgroup.com

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QA

Signed by: Estelle.Tressens@LGCGroup.com

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