

Zika Virus

NS1 AND ENVELOPE ANTIGENS & ANTIBODIES
FOR RAPID ASSAYS



The mosquito-borne Zika virus (ZIKV) is prompting worldwide concern due to its connection to a neurological birth disorder and its rapid spread across the globe. ZIKV appeared in Brazil in May 2015 and has since spread to 25 countries and territories. In February 2016, the World Health Organization (WHO) declared Zika virus a Public Health Emergency of International Concern.



ZIKA RECOMBINANT ANTIGENS

Recombinant Proteins

- Produced in insect cells
- Suitable for use in ELISA and rapid assays
- Detects both IgG and IgM antibodies to ZIKV

ZERO-X-REACT™ ZIKA ENVELOPE REC-ANTIGEN

NEW!

9050

- Mutated to eliminate cross-reactivity to the major conserved domain of other flaviviruses
- Contains a His tag

ZIKV NS1 PROTEIN

R01636

- NS1 is a large conserved protein believed to contain species of specific epitopes
- Contains a His tag

ZIKV ENVELOPE PROTEIN

R01635

- Sequence derived from the African strain (a.a. sequence is proprietary)
- The E protein comprises the majority of the virion surface and is involved in replication

ZIKA ANTIBODIES

Monoclonals

- Suitable for use in ELISA and Lateral Flow
- Produced *in-vivo*

MAB TO ZIKA NS1

C01864M	C01870M
C01865M	C01887M (Pair)
C01866M	C01888M (Pair)
C01867M (Pair)	C01889M (Pair)
C01868M (Pair)	C01890M (Pair)
C01869M	

MAB TO ZIKA ENVELOPE

C01860M
C01861M
C01862M
C01863M
C01938M

Polyclonals

- Produced in goat
- Suitable for use in ELISA and rapid assays

GOAT ANTI - ZIKA NS1

C01885G (Total IgG)

- Caprylic acid and ammonium sulfate fractionation

C01886G (Affinity Purified)

- Immunoaffinity purified by Zika NS1 conjugated Sepharose 4B Matrix

PRODUCT USAGE NOTES

There is an urgent medical need for rapid ZIKV diagnostic assays for use in the field to screen large numbers of samples. Existing tests include PCR assays and antibody (IgM/IgG) detection assays, however differentiation between flaviviruses remains challenging. Assays that directly test for the presence of ZIKV (e.g. PCR) are only able to detect infections up to 5 days after onset of symptoms – after this, the virus is no longer detectable. To overcome flavivirus cross-reactivity in diagnostics, recombinant antigens to envelope and NS1 proteins are commonly used. IgG and IgM antibodies typically show a high sensitivity and specificity to these epitopes, especially NS1 which is thought to contain more species specific epitopes than the envelope proteins. The biological safety level assigned to live Zika virus is BSL-2.

Protein Confirmation & Molecular Weight

The Zika antigens are expressed in insect cells and accordingly are expected to have the necessary post-translational modifications (e.g. glycosylation) required for proper folding. The native conformation of Zika NS1 is proposed to be hexameric, similar to other NS1 proteins, so molecular weight testing may produce varying results depending on the technique used (e.g. higher molecular weights may be seen using analytical SEC techniques due to oligomer formation). The specification for molecular weight provided on the COAs for the antigens were determined by reducing SDS-PAGE.

Reducing cross-reactivity with Dengue

To improve assay specificity, it is necessary to remove any cross-reacting antibodies that could bind to the antigen and cause a false result. Defined epitope blocking ELISAs have also been used to increase the specificity and have been useful for differentiating flaviviral infections through targeting epitopes on NS1 or E protein. By including low concentrations of unconjugated Dengue NS1 and Chikungunya NS1 antigens, it is possible to block antibodies that are highly cross reactive between the three flaviviruses.

RECOMMENDED DENGUE RECOMBINANTS:

R01656 (NS1, Type 1)	R01659 (Envelope, Type 1)
R01657 (NS1, Type 2)	R01660 (Envelope, Type 2)
R01658 (NS1, Type 3)	R01661 (Envelope, Type 3)
R01663 (NS1, Type 4)	R01662 (Envelope, Type 4)

Methods to increase IgM sensitivity

To improve assay sensitivity to IgM, we recommended the following:

- 1. IgM-Capture Assays:** Use anti-human IgM Fab fragment antibody as the capture as opposed to a full-length anti-human IgM antibody. This allows more Fab fragment antibodies to bind to the surface area on the solid substrate increasing the number of sites available for total IgM antibody to be captured.
- 2. Lateral Flow Assays:** Employ the bridging method in which colloidal gold-labelled disease specific antibody (e.g. MAb or PAb to Zika NS1) is pre-mixed with recombinant antigen (e.g. Zika NS1). Conjugating colloidal gold directly to the Zika antigen can inhibit its ability to bind to captured IgM. Furthermore using a gold-conjugated PAb, which has a broad reactivity, can further increase assay sensitivity. A PAb can bind to different antigen epitopes therefore enabling more than one PAb to bind to the same antigen simultaneously to generate a stronger signal.

RECOMMENDED ZIKA POLYCLONALS:

C01885G (*Total IgG*)

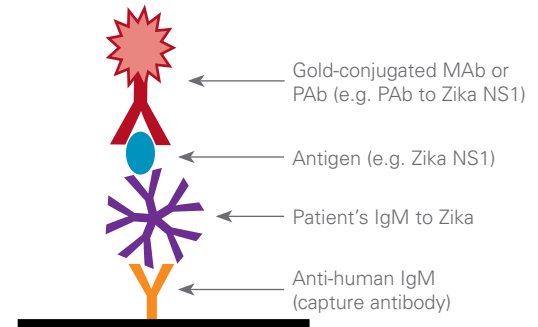
C01886G (*Affinity Purified*)

* C01886G Purified using Zika Virus NS1 to minimize cross-reactivity to other flaviviruses such as Dengue NS1

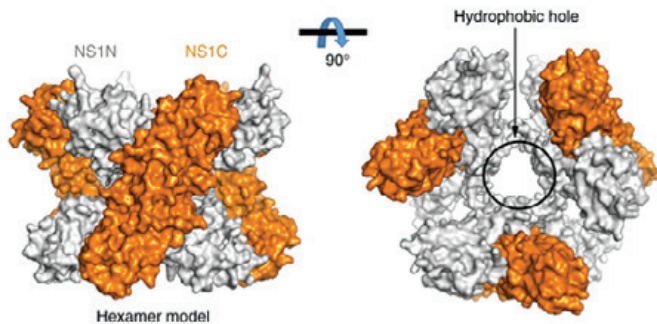


Protocol suggestions for using the bridging method to improve assay sensitivity and specificity:

- Conjugate the gold particle to the anti-Zika NS1 Ab (optimization of conjugation chemistry required).
- Mix the recombinant Zika NS1 antigen and gold-conjugated anti-Zika NS1 Ab to form a complex.
 - The ratio of Zika NS1 and gold-conjugated anti-Zika NS1 Ab must be optimized for a particular assay.
 - Remove any unbound gold-conjugated anti-Zika NS1 (e.g. via centrifugation) prior to deposition on the conjugate pad.
 - In order to reduce cross-reactivity with Dengue, include a recombinant Dengue NS1 antigen to conjugate mixture (inclusion of one serotype should be sufficient). Optimal dilution must be determined by the end user.



Model of the ZIKV NS1 hexamer



The ZIKV NS1 hexamer, modeled by using the full-length Dengue Type 2 NS1 structure. The actual NS1₁₇₂₋₃₅₂ structure is colored in orange, and the predicted NS1₁₋₁₇₁ structure is colored in light gray. NS1₁₇₂₋₃₅₂ is located in the outer layer of the hexamer barrel.

Zika NS1 shares a high structural similarity with other flavivirus NS1 proteins and NS1 is suspected to be a major genetic factor underlying the diverse clinical consequences of infections caused by flaviviruses.

Zika NS1 displays characteristics that are unique among flaviviruses including a loop surface containing both a positively and negatively charged central region and a negative charge toward the two distal ends in its loop surface. This loop-surface interface is thought to play a crucial role in interactions of secreted NS1 hexamers with host factors and antibodies.

Song, H., Qi, J, Haywood, J., Shi, Y. and Gao, G.F. (2016). *Zika virus NS1 structure reveals diversity of electrostatic surfaces among flaviviruses*. *Nature Structural & Molecular Biology*. 23:456–458.

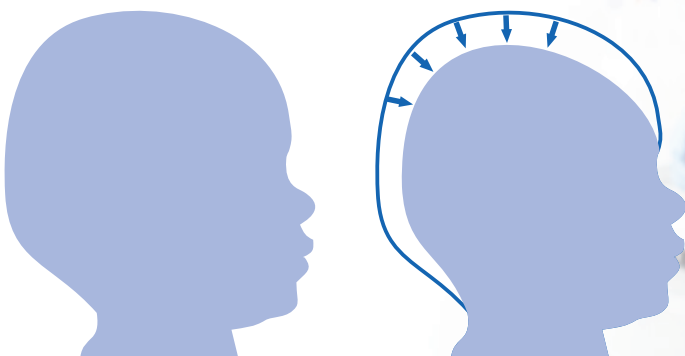


Fig. 1: Microcephaly: a neonatal malformation defined as a head size much smaller compared with other babies of the same age and sex.



ZIKA VIRUS - BACKGROUND INFORMATION

Zika (ZIKV) virus is a mosquito-borne flavivirus that was first identified in a monkey in Uganda in 1947. Outbreaks of ZIKV disease have been recorded in Africa, the Americas, Asia and the Pacific, however, ZIKV appeared in Brazil for the first time in May 2015 and since then has been estimated to have infected up to 1.3 million people and spread to 25 countries and territories. There is scientific consensus that Zika virus is a cause of microcephaly and Guillain-Barré syndrome. Links to other neurological complications are also being investigated.

ZIKV is closely related to other mosquito-borne flaviviruses such as Dengue, Japanese encephalitis, yellow fever, and West Nile. Structurally, like other flaviviruses, ZIKV is icosahedral shaped and enveloped with a non-segmented, single-stranded, positive-sense RNA genome which encodes seven nonstructural proteins and three structural proteins. The structural proteins encapsulate the virus and the replicated RNA strand is held within a nucleocapsid which is contained within a host-derived membrane modified with two viral glycoproteins. There are two lineages of Zika (African and Asian lineage) and phylogenetic studies indicate that the virus spreading in the Americas is 89% identical to African genotypes, but is most closely related to the Asian strain that circulated in French Polynesia during the 2013–2014 outbreak.

ZIKV is primarily transmitted by infected *Aedes* species mosquitos (*A. aegypti* and *A. albopictus*) although it appears that it can also be spread through blood transfusion and sexual contact. Control measures aim to minimize any exposure to potentially ZIKV infected mosquitos. However, given that the global distribution of *A. aegypti* is expanding due to global trade and travel (including continents such as North America and the European periphery) ZIKV will likely continue to spread to new areas. Before the current pandemic began, ZIKV was not known to spread widely among humans or cause any neurological complications however ZIKV does possess the ability to mutate rapidly. Sequence analysis has shown that that virus has undergone significant genetic changes in the past 70 years, including substantial DNA changes between the Asian and African lineages, as well as the human and mosquito strains. It has been suggested that these mutations could enable the viruses to replicate more efficiently, evade the body's immune response or invade new tissues that provide a safe harbor for it to spread.

In general, infection with Zika fever is a mild disease only causing a rash, fever, joint pain, and malaise, similar to Dengue fever. However, ZIKV infection during pregnancy can cause a serious birth defect called microcephaly, as well as other severe fetal brain defects. It has also been linked to other cases of several neurological complications including Guillain-Barré syndrome and hearing difficulties.

