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The NativeAntigen COMPANY

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Introduction

Dengue, a flavivirus transmitted primarily by *Aedes aegypti* mosquitoes, has been extensively studied as it is a significant threat to human health. Though infection is normally self-limiting with flu-like symptoms and high fever, Severe Dengue (Dengue Haemorrhagic Fever) is a serious and potentially fatal complication, and is a leading cause of hospitalisation and death throughout regions of Asia and Latin America.

In recent years the global impact of the disease has risen dramatically. **Now endemic in over 100 tropical and sub-tropical countries, Dengue is recognised as one of the biggest emerging disease threats worldwide.** In fact, the geographical spread is increasing rapidly, and estimates suggest that 3.9 billion people in 128 countries are now at risk of infection [1] – about half the global population. What's more, this extensive spread is associated with high prevalence, with approximately 100 million symptomatic and 300 million asymptomatic cases occurring annually [2].

The prevalence of Severe Dengue is also increasing, and it is now estimated that 500,000 people require hospitalization for this condition each year [3]. As such, it has become a major public health problem. Given the serious impact of Severe Dengue, it is important to understand the risk factors for this complication. There are four serotypes of Dengue virus and in many Dengue endemic areas two or more serotypes may be circulating at the same time. Primary infection with Dengue typically leads to mild illness and life-long immunity to that serotype, but a secondary infection with a different serotype has a high risk of causing Severe Dengue. To a large degree this is caused by the host's own immune response, by a phenomenon known as antibody-dependent enhancement [4].

There is no specific treatment for Dengue, so disease control relies heavily on prevention. The one vaccine that *is* clinically available has shown issues with safety, as it worsens the risk of Severe Dengue in seronegative individuals. **Given the serious human health risks and the profound socio-economic impact of the disease, there is an urgent need for academics and researchers in the biotechnology and pharmaceutical industries to work towards more effective control strategies.** To develop new diagnostic and pharmaceutical solutions, researchers need access to highly specific viral antigens and antibodies.

Challenges associated with Dengue prevention and control

Given the lack of specific therapies for Dengue, medical care is limited to supportive treatment only. If the disease is diagnosed early, care can reduce the fatality rate of Severe Dengue from over 20% to under 1%. **Therefore, since disease mortality is so dependent on early diagnosis, it is important to have accurate and reliable diagnostic tests available.**

To control disease spread, the main preventative strategies are vector control and vaccination. Vaccination presents significant challenges, however, because of the nature of immunity to the four different serotypes of Dengue.

The first vaccine licensed for Dengue, a live attenuated vaccine called Dengvaxia® (CYD-TDV), has proved safe and effective in seropositive people but increases the risk of Severe Dengue in seronegative individuals. Though Dengvaxia has been approved in 20 countries, its utility is limited due to these safety issues. Therefore, in order to mitigate patient risk, the World Health Organisation (WHO) recommends pre-vaccine serological screening to ensure that only seropositive patients are vaccinated with Dengvaxia.

Given the limitations of Dengvaxia, there is a real need for alternative vaccines that are safe and effective across a wider patient population. Unfortunately, no other vaccine options are currently available, though promising new candidates are now in Phase 3 clinical trials. **For new vaccine candidates to be successful, they must be safe in both seropositive and seronegative individuals and should protect against all four serotypes of Dengue. In order to successfully develop effective new vaccines and powerful serological tests, researchers must select and synthesise the most appropriate antigen targets.**

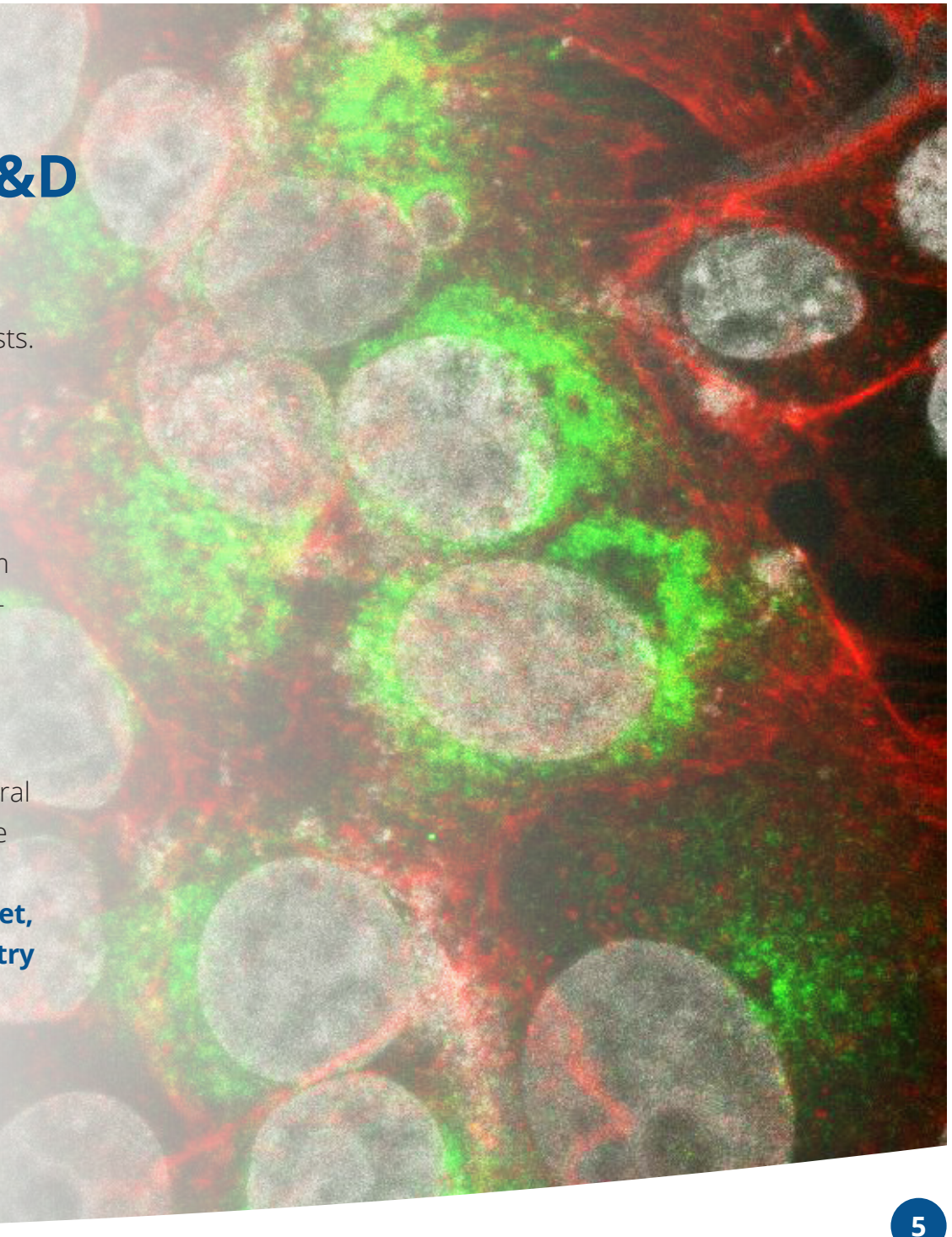
Key proteins in Dengue R&D

Studying the viral structure has helped identify the most promising antigen targets for new vaccines and diagnostic tests. Research to date has focused on two areas of interest:

Envelope and membrane proteins

The outer protein shell of a Dengue virion is composed of an envelope protein (E) and a membrane protein (M), which form M-E heterodimers on the mature virus. In immature and non-infectious viral particles, however, the precursor of M protein (prM) is found in a heterocomplex with E.

During infection, E binds a receptor on the host cell surface and undergoes a conformational change, which causes the viral membrane to fuse with the target cell membrane. E therefore plays an essential role in delivering the viral genome into the cytosol. As such, **Envelope proteins are a key vaccine target, as they can potentially be exploited in blocking virus entry to host cells**.



NS1 protein

Non-structural protein 1 (NS1) is also key, because it performs a range of important viral functions. Firstly, it is required for viral RNA replication and for producing infectious viral particles [5]. In addition to this function, it also plays an important role in pathogenesis, as it is secreted from virally infected cells to damage host endothelial cells. This damage is caused by effects on endothelial glycocalyx components [6], and results in endothelial cell hyperpermeability. The ability of the NS1 protein to exert these pathogenic effects depends on its structure as a barrel-shaped homohexamer.

Since NS1 is secreted by infected cells, the measurement of NS1 levels can be used as a diagnostic tool to indicate active infection. As such, NS1 has proved an important target for research and development.

To develop vaccines, therapeutics or diagnostics based on these protein targets, academics and industry researchers need access to high-quality viral proteins and highly specific antibodies. Recombinant proteins are preferred for these studies, due to the difficulty in handling native Dengue virus in culture. As such, for this research to deliver valuable insights, it's important that the proteins used are representative of native viral proteins in terms of their function and antigenicity. However, it can be difficult to synthesise proteins in their biologically active form.

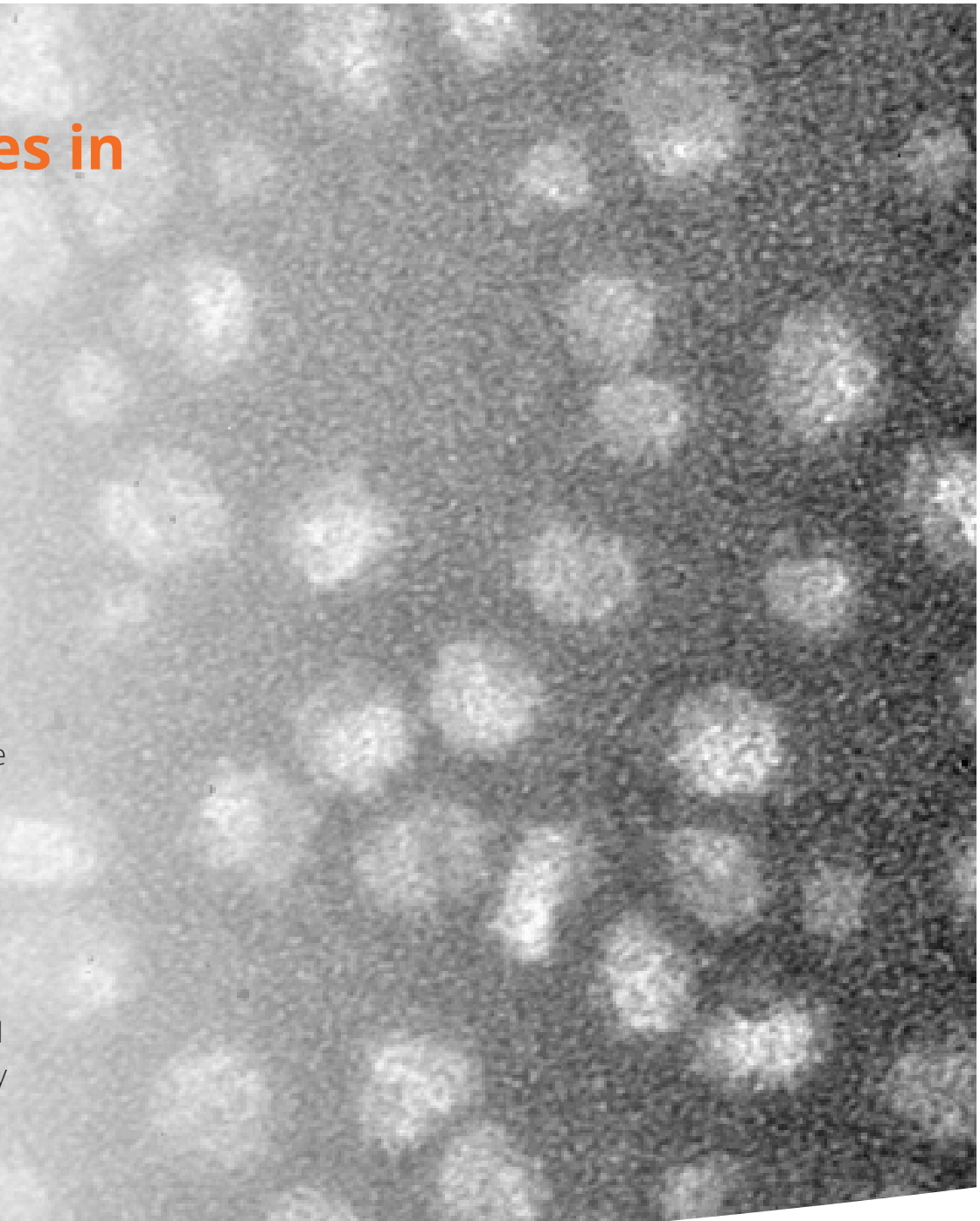
The challenge here is that the biological activity of these proteins depends on their structure, which can be difficult to reproduce with commonly used expression systems. The precise folding patterns and glycosylation are particularly critical to replicate, but there is no glycosylation in *E. coli* expression systems, and in insect systems the patterns of these post-translational modifications tend to differ. Therefore, the proteins produced may not be fully representative of native viral proteins in terms of their activity and antigenicity. This has been a major limitation in Dengue research, which has only recently been overcome.

Overcoming the challenges in Dengue research

Using a novel mammalian expression system, The Native Antigen Company (NAC) has developed recombinant Dengue proteins that are highly representative of native viral proteins. **Produced by human cells, these proteins are fully glycosylated, naturally folded and assembled, and have the same binding activity as the native proteins – making them ideal for use in your research and vaccine development studies.**

This unique expression system has been used to develop a range of cutting-edge products, including the world's first mammalian expressed Dengue NS1 proteins. Also available are recombinant Envelope proteins and virus-like particles (VLPs). These recombinant protein products have also been utilised as immunogens to prepare a new range of Dengue-specific antibodies.

These proteins and highly specific antibodies have the potential to help academics and biotechnology/pharmaceutical companies drive forward research and development, ultimately bringing more tests and vaccines to clinical use. The following pages will discuss these products in more detail.

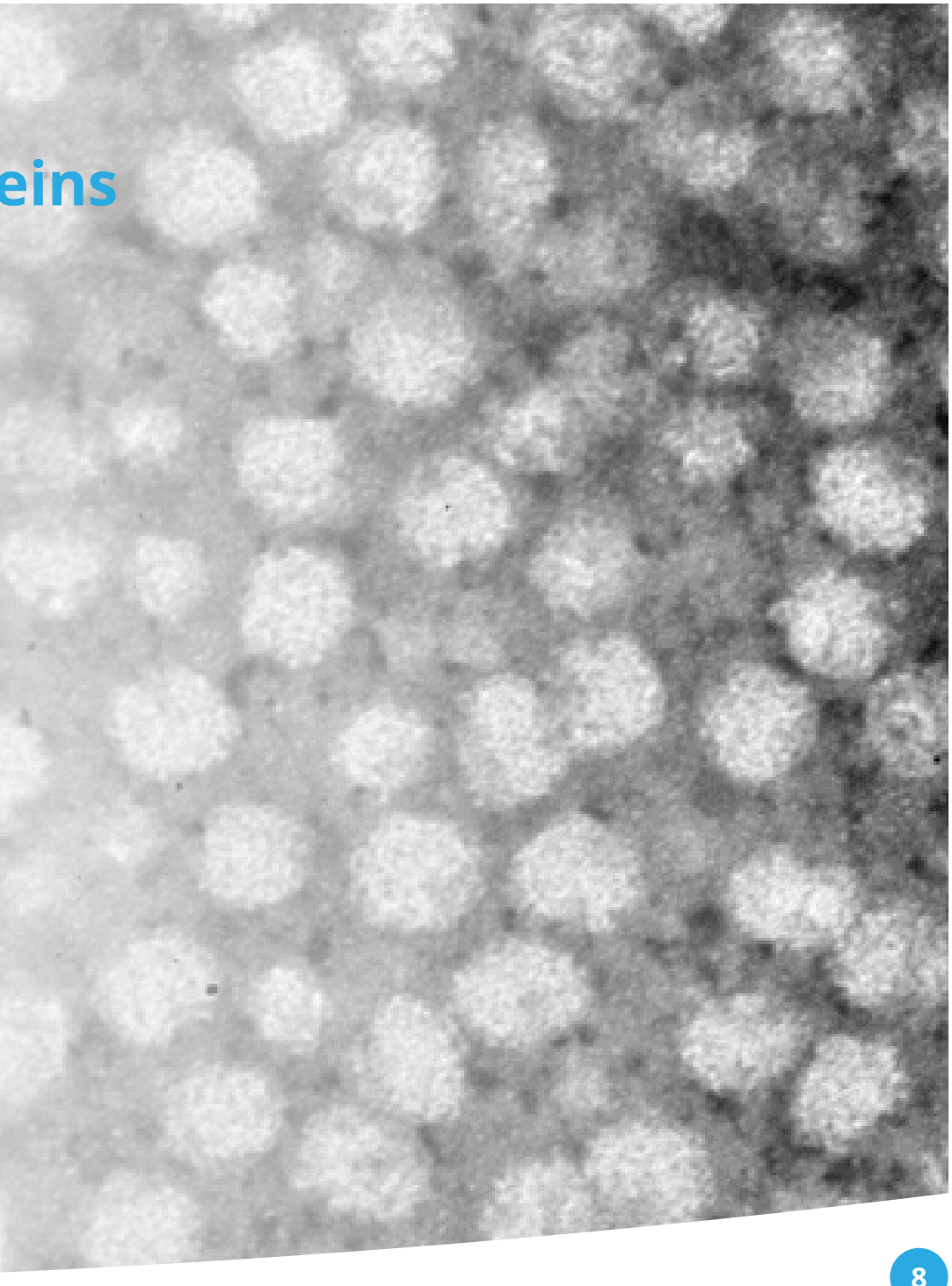


Recombinant Dengue proteins

The recombinant Dengue proteins produced in NAC's novel development system:

- **Exhibit optimal antigenicity due to their human origin**
- **Are in their biologically active form – fully folded, with all post-translational modifications**
- **Are highly purified and concentrated**

The two recombinant proteins available to support your research are Envelope protein and NS1. As catalogue products, these are available as wild-type forms derived from commonly used laboratory strains of each Dengue virus serotype. However, we can also customise these key proteins with specific mutations, or derive them from alternative strains. For example, we can produce mutants to examine the effect of glycosylation sites, or prepare proteins from Dengue strains relevant to particular countries. As we have the capacity to customise these products, we can alter them to suit your individual research aims, allowing you to effectively de-risk and optimise each project.



NS1 proteins

Given the key role of NS1 in viral replication and pathogenesis, these recombinant proteins offer powerful tools to study Dengue virus biology, permitting functional studies of protein activity both in cell culture and in animal models. What's more, as NS1 proteins are found at high levels in the bloodstream during active infection, they are often used as diagnostic biomarkers. Also, given that most current vaccine candidates focus on Envelope antigens, assays for NS1 proteins are important in distinguishing responses to natural infection and to vaccination.

Our recombinant NS1 proteins are available for all four Dengue serotypes, and are ideal as control reagents in antigen assays. It is critically important that these antigens are representative of the native viral proteins, since the pathogenic function of NS1 depends upon its homohexameric structure.

Envelope proteins

These proteins have been widely studied to investigate their role in cellular binding and infection. To help advance future studies, our mammalian expressed Envelope proteins are available for all four Dengue serotypes. As their structures are highly representative of the native proteins, these recombinant proteins can be used to advance research into Envelope protein, determining which specific domains are important for the immune response and which are important for viral entry into host cells. Such studies could guide further pharmaceutical development.

Virus-like particles

Complementing the soluble proteins described above, NAC also produces recombinant Dengue virus-like particles (VLPs). Consisting of shells of outer viral proteins, recombinant VLPs are an emerging vaccine technology. The key advantage of VLPs is that they are highly immunogenic, as their structure is representative of how viral antigens are presented *in vivo*, but they are non-infectious as they lack the core genetic material. Each recombinant VLP consists of 180 copies of E protein, combined with prM/M protein to form a viral particle.

A recent study [7] suggests that NAC's Dengue VLPs **provide a safe and practical alternative to the infectious virus for the study of vaccines and for developing diagnostic tests.** These products will therefore help drive research into new clinical applications. All four serotypes are available, providing you with a useful tool to aid thorough research studies and product development.

Our VLPs are produced in human cell lines using our proprietary advanced expression system. They are highly concentrated and purified in order to help ensure good data integrity and reproducibility – enabling your research and development projects to run smoothly, on time and on budget.

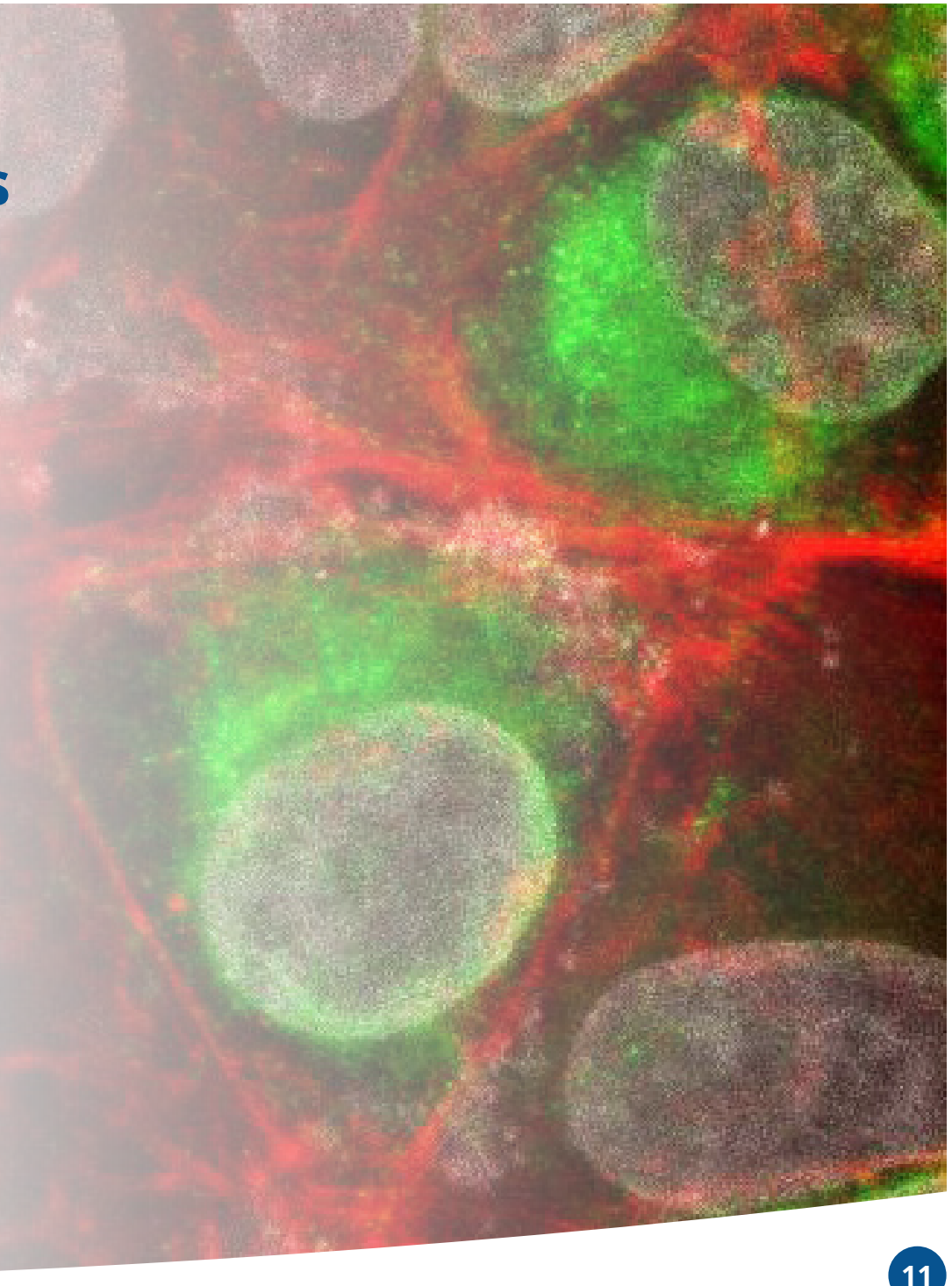
PROTEIN DESCRIPTION	EXPRESSION SYSTEM	TAG	PRODUCT CODE
Dengue virus serotype 1 NS1 protein	MAMMALIAN	HIS	DENV1-NS1
Dengue virus serotype 2 NS1 protein	MAMMALIAN	HIS	DENV2-NS1
Dengue virus serotype 3 NS1 protein	MAMMALIAN	HIS	DENV3-NS1
Dengue virus serotype 4 NS1 protein	MAMMALIAN	HIS	DENV4-NS1
Dengue virus serotype 1 VLP	MAMMALIAN	NONE	DENV1-VLP
Dengue virus serotype 2 VLP	MAMMALIAN	NONE	DENV2-VLP
Dengue virus serotype 3 VLP	MAMMALIAN	NONE	DENV3-VLP
Dengue virus serotype 4 VLP	MAMMALIAN	NONE	DENV4-VLP
Dengue virus serotype 1 Envelope protein	MAMMALIAN	HUMAN FC	REC31635
Dengue virus serotype 2 Envelope protein	MAMMALIAN	HIS	DENV2-ENV
Dengue virus serotype 3 Envelope protein	MAMMALIAN	HIS	REC31625
Dengue virus serotype 3 Envelope protein	MAMMALIAN	HUMAN FC	REC31636
Dengue virus serotype 3 Envelope protein	MAMMALIAN	MOUSE FC	REC31650
Dengue virus serotype 4 Envelope protein	MAMMALIAN	HIS	DENV4-ENV
Dengue virus serotype 1 Envelope protein	INSECT	HIS	REC31679
Dengue virus serotype 2 Envelope protein	INSECT	HIS	REC31680
Dengue virus serotype 3 Envelope protein	INSECT	HIS	REC31681
Dengue virus serotype 4 Envelope protein	INSECT	HIS	REC31682

Dengue-specific antibodies

The generation of high-quality antibodies ultimately depends on using high-quality antigens. At NAC, we have used the recombinant proteins described above as antigens to raise a unique panel of Dengue virus-specific monoclonal antibodies. Given the significant structural similarities between flaviviruses and the potential for cross-reactivity, Dengue antibodies must be **highly sensitive and specific for use in diagnostic immunoassays.** Therefore, we have utilised our extensive panel of flavivirus proteins to screen and select antibodies of the highest specificity.

Antibodies must also be carefully characterised for their use in a range of applications, including ELISA, Western Blotting and Immunofluorescence. It is unusual for a particular antibody to perform well in all applications, so good characterisation of each reagent is key. Therefore, we have tested our antibodies in a range of applications and determined their sensitivity and specificity, so that we can provide you with advice on which antibody is best suited to your application.

The panel of high-quality antibodies available includes both mouse and human anti-NS1 for different serotypes, as well as anti-Envelope protein antibodies.



SPECIFICITY	HOST	SUB-CLASS	PRODUCT CODE	APPLICATIONS
Dengue virus serotype 2	MOUSE	IgG1	MAB12135	E, IF
Dengue virus envelope protein (pan-serotype)	MOUSE	IgG2a	MAB12267	E, WB
Dengue virus NS1 (pan-serotype)	MOUSE	IgG1	ABDENVNS1-DA034	E, IF
Dengue virus NS1 (serotype 1)	MOUSE	IgG1	MAB12132	E, WB
Dengue virus NS1 (serotype 1)	HUMAN (CHIMERIC)	IgG1	MAB12261	E
Dengue virus NS1 (serotype 1)	HUMAN (CHIMERIC)	IgM	MAB12262	E
Dengue virus NS1 (serotype 2)	MOUSE	IgG2b	MAB12133	E, WB
Dengue virus NS1 (serotype 2)	MOUSE	IgG1	MAB12134	E, IF
Dengue virus NS1 (serotype 2)	MOUSE	IgG	ABDENV2NS1-CM474	E, IF
Dengue virus NS1 (serotype 2)	MOUSE	IgG	ABDENV2NS1-CM435	E
Dengue virus NS1 (serotype 3)	MOUSE	IgG1	MAB12149	E
Dengue virus NS1 (serotype 4)	MOUSE	IgG	ABDENV14NS1-CM436	E
Dengue virus NS1 (serotype 4)	MOUSE	IgG1	MAB12179	E
Dengue virus prM protein (serotypes 1-4)	MOUSE	IgG1	MAB12136	E, IF

APPLICATIONS: E=ELISA WB=WESTERN BLOT IF=IMMUNOFLUORESCENCE

Looking ahead

Dengue virus presents a significant and growing threat to human health. As such, there is a real need for improved vaccines and serological tests to help combat the rapid spread of this serious disease. To drive the development of these new products, academics and industry researchers need access to high-quality antibodies and antigens.

Developed using a novel advanced expression system, NAC's high-quality proteins, VLPs, and highly specific antibodies now provide cutting-edge solutions to accelerate Dengue research and development. A key advantage of these products is that they can be tailored to support your specific research application. For example, proteins can be customised for specific assays and we can also provide you with advice and expertise on choosing optimised reagents depending on your individual circumstances. In this way, these solutions will help your research project to run in good time- and cost-efficiency.

Advancing Dengue research with these customisable proteins will help academics ensure the quality of their experimental data, raising the likelihood of obtaining funding and publication. It will also help biotechnology and pharmaceutical companies to develop effective new tests and vaccines. As such, these highly specific products should prove invaluable tools in the global battle against Dengue.

To learn more about the novel solutions available to help accelerate your Dengue research, please visit **www.thenativeantigencompany.com/dengue-virus**

References

1. O. Brady, P. Gething, S. Bhatt, J. Messina, J. Brownstein, A. Hoen, C. Moyes, A. Farlow, T. Scott, and S. Hay, "Refining the global spatial limits of dengue virus transmission by evidence-based consensus," PLoS Negl. Trop. Dis., vol. 6, no. 8, p. 1760, 2012.

2. S. Bhatt, P. W. Gething, O. J. Brady, J. P. Messina, A. W. Farlow, C. L. Moyes, J. M. Drake, J. S. Brownstein, A. G. Hoen, O. Sankoh, M. F. Myers, D. B. George, T. Jaenisch, G. R. W. Wint, C. P. Simmons, T. W. Scott, J. J. Farrar, and S. I. Hay, "The global distribution and burden of dengue," Nature, vol. 496, p. 504, Apr. 2013.

3. "WHO factsheet on Dengue: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue." .

4. S. B. Halstead, "Dengue Antibody-Dependent Enhancement: Knowns and Unknowns," Microbiol. Spectr., vol. 2, no. 6, Dec. 2014.

5. P. Scaturro, M. Cortese, L. Chatel-Chaix, W. Fischl, and R. Bartenschlager, "Dengue Virus Non-structural Protein 1 Modulates Infectious Particle Production via Interaction with the Structural Proteins," PLOS Pathog., vol. 11, no. 11, p. e1005277, Nov. 2015.

6. D. R. Glasner, K. Ratnasiri, H. Puerta-Guardo, D. A. Espinosa, P. R. Beatty, and E. Harris, "Dengue virus NS1 cytokine-independent vascular leak is dependent on endothelial glycocalyx components," PLOS Pathog., vol. 13, no. 11, p. e1006673, Nov. 2017.

7. S. W. Metz, A. Thomas, L. White, M. Stoops, M. Corten, H. Hannemann, and A. M. de Silva, "Dengue virus-like particles mimic the antigenic properties of the infectious dengue virus envelope," Virol. J., vol. 15, no. 1, p. 60, Dec. 2018.