NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit

Global monitoring of influenza strains for resistance to antiviral inhibitors is essential for studying epidemiology of viral strains and mutations and for reliably understanding the efficacy of antiviral therapeutics in the event of a significant influenza outbreak. Neuraminidase inhibitor drugs are the primary anti-influenza therapeutics that will be relied upon to contain a potential outbreak.

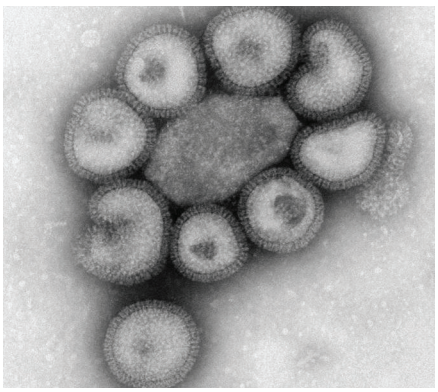

Figure 1. Influenza Viral Particles Attached to a Cell.

In consultation with worldwide public health protection agencies, including the Centers for Disease Control (CDC) and member laboratories of the Neuraminidase Inhibitor Susceptibility Network (NISN), Applied Biosystems' 1,2-dioxetane chemiluminescent technology has been combined to form the NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit to measure the level of neuraminidase inhibitor resistance in influenza virus isolates. In addition to getting reliable and consistent results through the use of standardized reagents and protocols, these kits offer:

- High sensitivity for detection of low virus concentrations in a wide range of samples
- Broad specificity for quantitation of multiple virus types
- Reliable availability of assay reagents

This highly sensitive, rapid, and standardized detection assay can be used to quantitate the level of neuraminidase inhibitor resistance of virus isolates from many sources and cultured by multiple methods.

A Comprehensive Offering

The Applied Biosystems® NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit provides all of the reagents necessary for complete assay performance, 96-well solid white microplates, and a comprehensive protocol. The key reagent component is the NA-*Star*® chemiluminescent substrate for neuraminidase, sodium (2-chloro-5-(4-methoxyspiro{1,2-dioxetane-3,2′-(5-chloro)tricyclo[3.3.1.1^{3,7}]decan}-4-yl-phenyl 5-acetamido-3,5-dideoxy-α-D-glycero-D-galacto-2-nonulopyranoside) onate. A single buffer, NA-*Star*® Assay Buffer, is the diluent for virus samples, neuraminidase inhibitors, and NA-*Star*® substrate. The NA-*Star*® Accelerator solution triggers high-intensity light emission from the reaction product, immediately upon addition.

In addition to the reagent components, the kit includes NA-*Star*® Detection Microplates, 96-well solid white assay microplates. These plates were selected for optimum assay performance, including high signal intensity, low background, and minimum well-to-well crosstalk. The kit also includes a comprehensive assay protocol, providing virus culture dilution recommendations, instructions for serial dilution of neuraminidase inhibitors (NI), a recommended plate layout with necessary assay control wells, a detailed step-by-step assay protocol, and a complete literature reference list.

The complete reagent set, provided ready-to-use with a single, simple substrate dilution step together with assay microplates and protocol, permits facile assay performance by a wide range of laboratories. Each kit provides sufficient reagents for performing ten 96-well plate assays (960 assay wells), providing the capacity to simultaneously assay multiple virus isolates with multiple neuraminidase inhibitors.

The NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit has been verified for performance on multiple dedicated luminometer instruments, using both on-board reagent injection and manual reagent addition with a multichannel pipettor. The NA-*Star*® detection assay is compatible with a wide range of luminometer instrumentation typically available in many laboratories.

High Sensitivity and Wide Assay Range

Applied Biosystems' 1,2-dioxetane chemiluminescent substrate technology has been widely proven to provide highly sensitive enzyme detection in many different biomolecule detection applications, enabling superior assay sensitivity compared to fluorometric or spectrophotometric detection.

The NA-*Star*® chemiluminescent substrate provides higher detection sensitivity (low-end detection limit), higher assay signal-to-noise, and wider assay dynamic range than fluorescent assays with the methylumbelliferyl *N*-acetylneuraminic acid (MUNANA) substrate. These detection technologies have been compared in assays using both purified bacterial neuraminidase enzymes and many influenza virus isolates, both in internal assay development and by numerous research laboratories. Up to 50-fold higher sensitivity is achieved with NA-*Star*® assay detection. In addition, the NA-*Star*® substrate provides a dynamic range of detection of 3–4 orders of magnitude, compared to 1–2 orders of magnitude with the fluorescent MUNANA substrate.

Accurate determination of neuraminidase inhibitor IC_{50} values is achieved over a range of virus dilutions, eliminating the need to "titer" and adjust virus dilution prior to assay performance. Assay comparisons performed by several laboratories have shown good correlation between IC_{50} values obtained with the chemiluminescent NA-*Star*® assay and the MUNANA fluorescence assay.

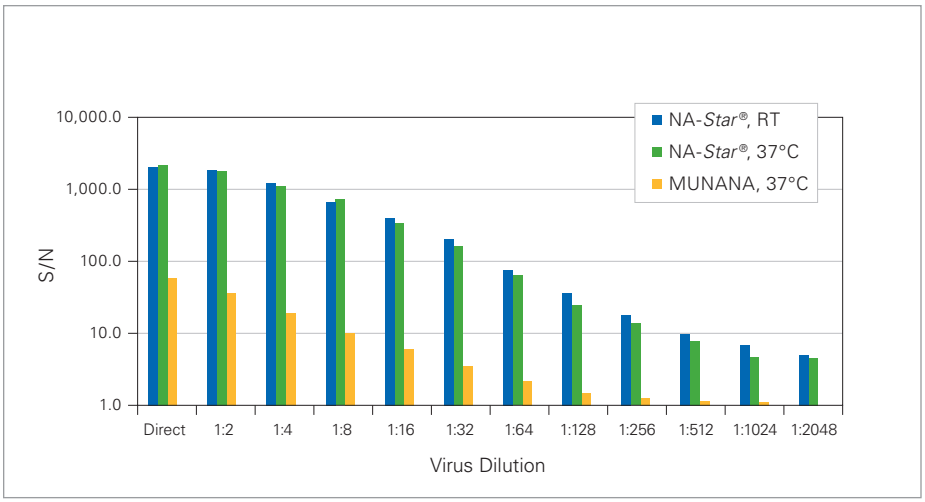

Figure 2. Comparison of Sensitivity of Chemiluminescence Detection With NA-*Star*® Substrate to Fluorescence Detection with MUNANA Substrate. Dilutions of influenza type B (ATCC VR-1535) virus culture supernatant (cultured on MDCK cells) were assayed at different temperatures, and the signal-to-noise (S/N) ratios were calculated using uninfected MDCK cell supernatant (noise). The lower limit of detection (S/N = 2) is at least 30-fold lower with the chemiluminescent NA-*Star*® assay, and the S/N is approximately 50-fold higher with the NA-*Star*® assay. The dynamic range of detection with the NA-*Star*® assay with virus samples is 3 orders of magnitude.

Broad Assay Capability

Influenza neuraminidase assays using NA-*Star*® substrate have been widely validated alongside fluorescent MUNANA assays with many different influenza strains. Testing on a wide range of isolates has been conducted at research laboratories in the US, Canada, Australia, Japan, Europe, and the UK. The NA-*Star*® chemiluminescent substrate provides highly sensitive detection of neuraminidase enzyme activity from many animal influenza viruses, including human types A and B, avian, equine, and porcine viruses. This broad assay capability makes it an important new tool for researching the global spread of drug resistance of influenza in humans, migratory birds, and livestock. The detection kit has additional assay applications, including screening and development of new neuraminidase inhibitors, viral quantitation for vaccine research and development, and neuraminidase quantitation in other viruses and bacteria.

Designed for Reliability and Consistency

Applied Biosystems has a long history of development and manufacturing of 1,2-dioxetane enzyme substrates, chemiluminescence enhancers, substrate formulations, and complete reagent kits. Each of these is manufactured and quality-tested under rigorous quality systems that enable reliable provision of our 1,2-dioxetane chemiluminescent substrates and reagents to multiple markets.

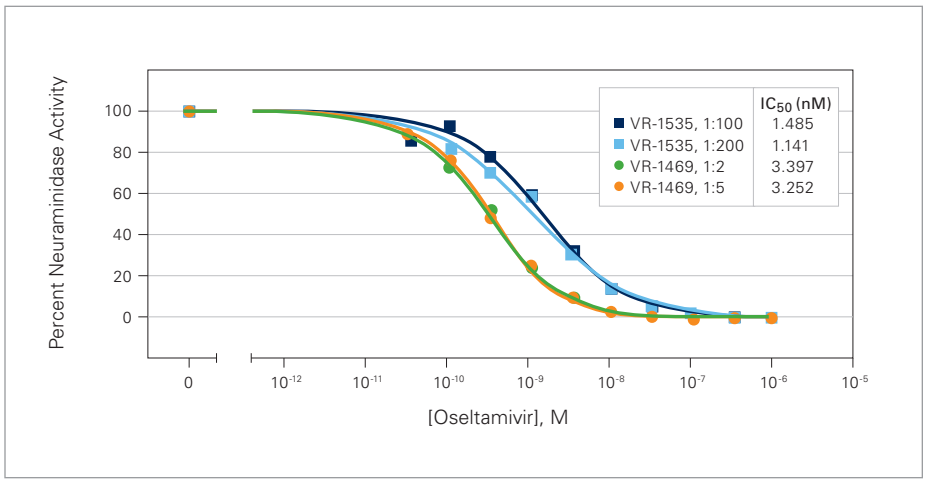

Figure 3. IC_{50} Determination With NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit. A half-log dilution series of oseltamivir, spanning 1,000–0.03 nM, was incubated with different dilutions of virus culture supernatants and assayed with the NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit. VR-1469 (influenza A/H1N1, strain A/PR/8/34, ATCC) and VR-1535 (influenza B, strain B/Lee/40) were cultured on MDCK cells.

Table 1. Influenza Strains Tested With NA-*Star*® Substrate.

Human	Avian	Equine	Porcine
A/H1N1	A/H2N5	A/H3N8	A/H7N1
A/H1N2	A/H2N9	A/H7N1	
A/H3N2	A/H3N8	A/H7N7	
B (many)	A/H5N1		
	A/H5N3		
	A/H7N1		
	A/H7N7		
	A/H8N4		
	A/H11N6		

Worldwide Support

To help our customers derive the most value from our technology and detection assays, we provide responsive, knowledge-able applications consulting, support, and technical service.

For more information, please contact your local Life Technologies sales representative, or go to **www.appliedbiosystems.com/support/contact**.

Table 2. NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit: Configuration And Required Instrumentation.

Kit components:

NA-*Star* ® Substrate
NA-*Star* ® Assay Buffer (used for diluting virus samples, NIs, and substrate)
NA-*Star* ® Accelerator
NA-*Star* ® Detection Microplates: 96-well white microplates
NA-*Star* ® Influenza Neuraminidase Inhibitor Resistance Detection Kit User Protocol

Demonstrated limit of detection:

1 x 10^{-4} U neuraminidase (*C. perfringens*)

Positive/negative reaction control:

Not included.

Purified bacterial neuraminidases (sialidases) can be obtained through several commercial sources for use as positive controls for reagent/instrument testing (if desired).

Neuraminidase inhibitor–resistant reference influenza strains can be obtained (if desired) through the CDC or other influenza reference laboratories. NI-sensitive reference influenza strains can be obtained through ATCC or influenza reference laboratories.

Required instrumentation:

Microplate luminometer (or multimode instrument with luminescence capability), ideally equipped with an on-board reagent injector. A luminometer without injection can be used if a multichannel pipettor is available, although the resulting signal intensity will be lower.

Data analysis software:

Not included.

Dose response (nonlinear curve fit) analysis software (ie., GraphPad Prism®) required for IC_{50} determination.

ORDERING INFORMATION

Description	Size	Part Number
NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit	960 assay wells + 10 x 96-well microplates	4374422
NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Reagent Set (does not include microplates)	960 assay wells	4374348
NA-*Star*® Detection Microplates	10 x 96-well microplates	4374349

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