Applied Biosystems® NA-XTD™ and NA-Fluor™ Influenza Neuraminidase Assay Kits

Global monitoring of influenza strains for resistance to neuraminidase inhibitors is essential for understanding their efficacy for seasonal, pandemic, or avian influenza, and for studying the epidemiology of viral strains and resistance mutations.

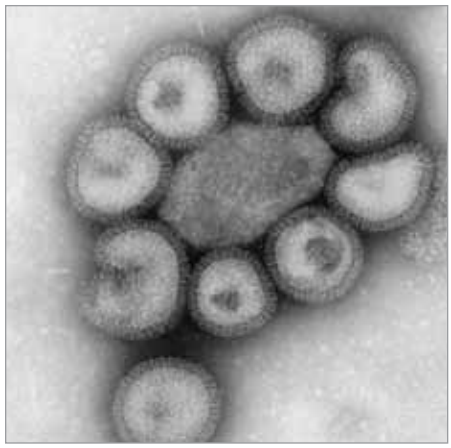


Figure 1. Influenza viral particles attached to a cell.

Life Technologies has developed two new influenza neuraminidase assay kits primarily designed for quantitation of neuraminidase inhibitor resistance. The Applied Biosystems® NA-XTD™ Influenza Neuraminidase Assay Kit is a next-generation chemiluminescence-based assay that provides a longer signal readout compared to the first-generation NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit. The Applied Biosystems® NA-Fluor™ Influenza Neuraminidase Assay Kit is a fluorescent assay based on the traditional 2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) substrate. These assay kits provide detection reagents with complete assay protocols for quantitating sensitivity of influenza virus isolates to neuraminidase inhibitors. Together, these kits offer:

- Reliable and consistent results through standardized reagents and protocols
- A choice of chemiluminescent or fluorescent detection technology
- Cost-effective, easy-to-use assays with simple instrumentation requirements
- High sensitivity for detection of low virus concentrations
- Batch-mode processing and compatibility with large-scale assay throughput
- Data comparable to historical susceptibility screening data
- Broad specificity for monitoring seasonal influenzas: human types A and B, A/H1N1 pandemic, avian, equine, and porcine
- Flexibility in assay format and assay run time
- Additional neuraminidase (NA) assay applications: cell-based viral assays, screening and characterization of new NA inhibitors

NA-XTD™ Influenza Neuraminidase Assay Kit

The NA-XTD™ Influenza Neuraminidase Assay Kit provides the NA-XTD™ chemiluminescent neuraminidase substrate, together with necessary assay reagents and optional microplates, to measure neuraminidase activity of influenza virus, either for neuraminidase inhibitor (NI) sensitivity with virus isolates, or for cell-based virus replication or inhibition assays. The NA-XTD chemiluminescent substrate is similar to the first-generation NA-*Star*® substrate, with a single structural change that provides a much longer-lasting chemiluminescent signal, with typically higher detection sensitivity. The NA-XTD™ Assay Buffer is used as diluent for virus samples, neuraminidase inhibitors, and NA-XTD substrate. An optional NA Sample Prep Buffer is also included for Triton® X-100 detergent addition to virus preparations, which increases NA activity in some virus preparations. The next-generation NA-XTD™ Accelerator solution triggers high intensity light emission from the NA-XTD reaction product.

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 and NI dilution recommendations, a recommended plate layout for NI sensitivity assays, a protocol for a 96-well cell-based virus quantitation assay, and a literature reference list.

The complete reagent set—provided ready-to-use with a single, simple substrate dilution step—together with assay microplates and protocol, allow for easy performance of assays by a wide range of laboratories. The kit provides sufficient reagents for performing 10 x 96-well microplate assays (960 assay wells).

The NA-XTD™ Influenza Neuraminidase Assay Kit is compatible with a wide range of luminometer instrumentation, including single-mode and multi-mode instruments, without the need for onboard reagent injectors.

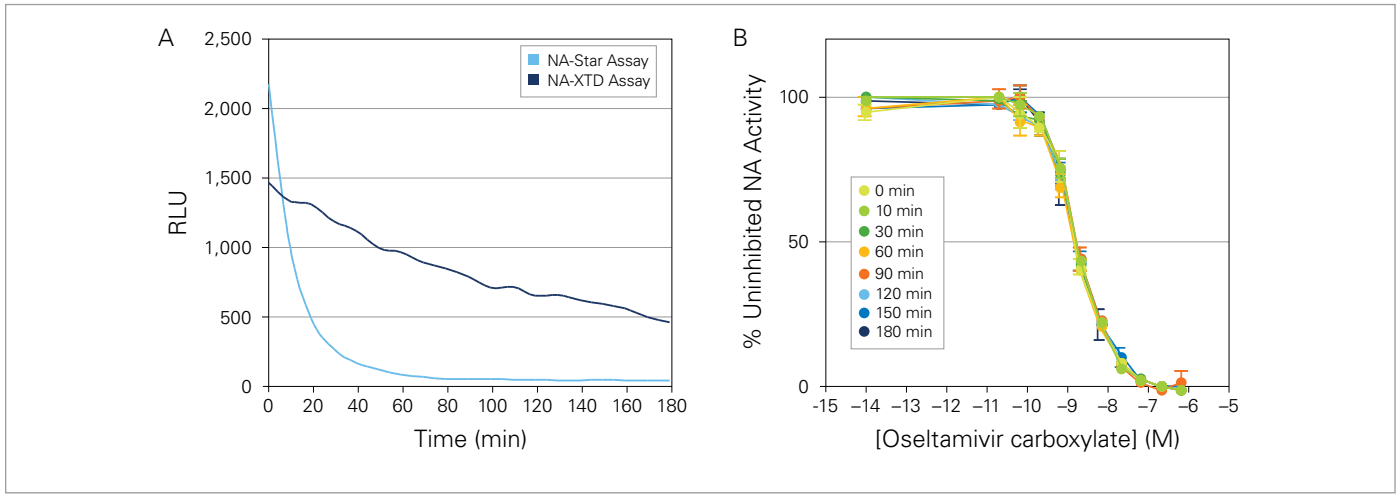


Figure 2. NA-XTD™ Assay Extended Light Emission Kinetics Enables Read-Time Flexibility. (A) The half-life of light emission following addition of NA-XTD™ Accelerator (T = 0) with the NA-XTD™ assay is ~2 hours, compared to ~10 minutes with the NA-*Star*® assay, using influenza A/WS/33 (H1N1) (VR-1520™, ATCC). **(B)** IC_{50} values were determined using data collected up to 3 hours after addition of NA-XTD™ Accelerator. The IC_{50} curves and values are identical at each time point using influenza B/Lee/40 (VR-1535™, ATCC).

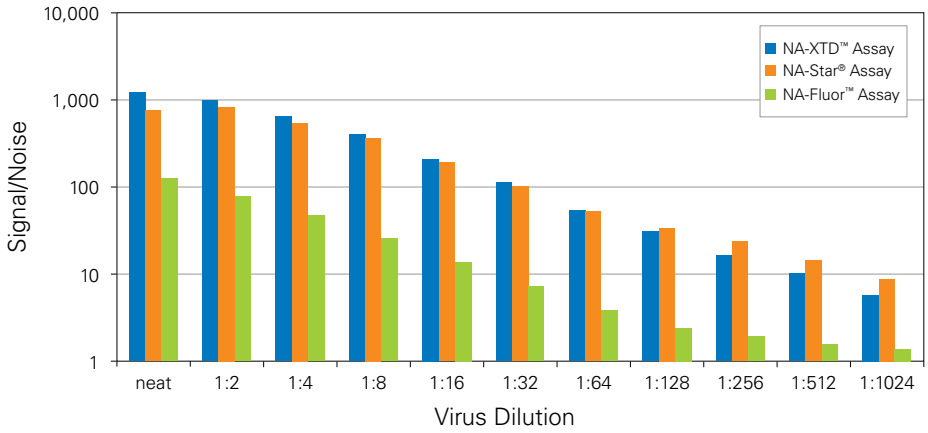


Figure 3. Sensitivity Comparison of NA-XTD™, NA-*Star*®, and NA-Fluor™ Assays. Serial 1:2 dilutions of influenza virus (A/WS/33 (H1N1) VR-1520™, ATCC) were assayed with each assay. A wide assay range is achieved with the chemiluminescent assays, with higher sensitivity than the fluorescent assay.

Long-Lived Light Emission Kinetics

The NA-XTD™ assay provides much longer-lived light emission kinetics than does the NA-*Star*® assay, eliminating the need for luminometer instrumentation with reagent injectors and enabling read-time flexibility and batch-mode processing of assay plates. The half-life of light emission achieved with the NA-XTD™ assay is ~2 hours, compared to 5–10 minutes with the NA-*Star*® assay. IC_{50} values determined from data collected immediately or up to 3 hours following addition of NA-XTD™ Accelerator solution are identical (Figure 2).

High Sensitivity and Wide Assay Dynamic Range

The NA-XTD™ chemiluminescent assay provides higher detection sensitivity (low-end detection limit), higher assay signal-to-noise, and a wider assay dynamic range than fluorescent assays with the MUNANA substrate. In addition, the NA-XTD™ assay typically demonstrates slightly higher signal-to-noise than the NA-*Star*® assay. The NA-XTD™ assay provides 2- to 50-fold higher sensitivity by signal-to-noise ratio than MUNANA-based fluorescence assays, depending on the virus isolate (Figure 3).

The NA-XTD™ assay, like the NA-*Star*® assay, provides a dynamic range of detection of 3–4 orders of magnitude of NA concentration, compared to 2–3 orders of magnitude range with fluorescent MUNANA assays. The wide assay range enables determination of IC_{50} values over a range of virus concentrations, eliminating the need to titer virus prior to performing IC_{50} determination assays. IC_{50} values obtained with the NA-XTD™ assay are similar to those obtained with the NA-*Star*® assay(Figure 4).

Cell-Based Virus Quantitation

The NA-XTD ™ assay can be used for virus quantitation in media samples from 96-well microplate or other virus cultures for monitoring viral growth or infection, or for performing viral inhibition assays in a cell-based system. Optimally, a small sample of culture media is removed and assayed directly with NA-XTD™ reagents, permitting multiple samples to be assayed over time (Figure 5).

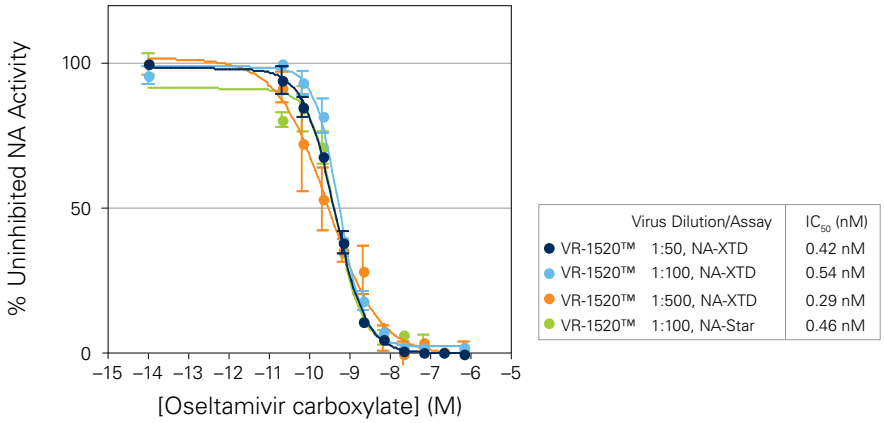


Figure 4. NA-XTD™ Assay IC_{50} Determination at Different Virus Dilutions. Oseltamivir carboxylate IC_{50} inhibition assay was performed with a range of virus dilutions (A/WS/33 (H1N1) VR-1520™, ATCC). IC_{50} values are nearly identical over a 10-fold difference in virus concentration.

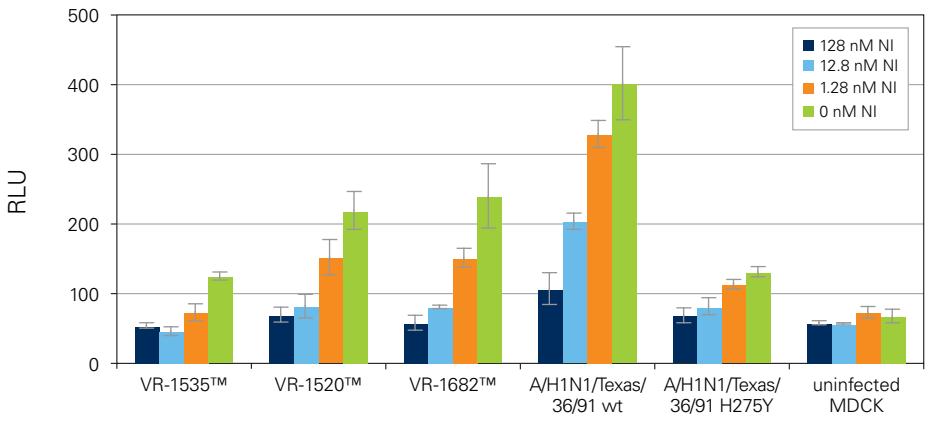


Figure 5. NA-XTD™ Assay Cell-Based Oseltamivir Inhibition. MDCK cell cultures in 96-well microplate were infected with different virus strains (B/Lee/40, VR-1535™, ATCC); A/WS/33 (H1N1), VR-1520™, ATCC); A/Swine/1976/31 (H1N1), VR-1682™, ATCC); A/Texas/36/91 (H1N1) wild-type and H275Y, kindly provided by the CDC)), followed by incubation in the presence of oseltamivir carboxylate. Samples of culture media were assayed 24 hours later. Quantitation of NA activity with the NA-XTD™ assay demonstrates inhibition of viral growth by oseltamivir in cell culture.

NA-Fluor™ Influenza Neuraminidase Assay Kit

The NA-Fluor™ Influenza Neuraminidase Assay Kit provides a standardized protocol and validated reagents for conducting neuraminidase enzyme assays, including neuraminidase inhibitor susceptibility screening. The assay reagents and protocol have been optimized based on NISN protocols to provide an economical fluorescent assay kit that is easy to use, generates data comparable to historical susceptibility screening data sets, is highly reliable, and contains environmentally friendly reagents.

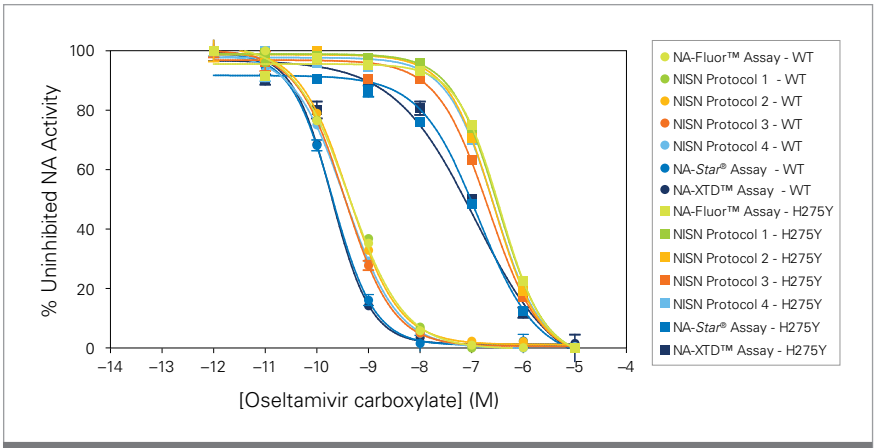
The kit includes comprehensive protocols for both titering viral isolates based upon neuraminidase activity and conducting neuraminidase enzyme inhibition assays. The assay can also be used for monitoring neuraminidase enzyme activity from non-viral sources. The kit provides sufficient reagents for performing ten 96-well assay microplates. Reagents include the fluorescent neuraminidase substrate, MUNANA, assay buffer, and stop solution, which is used to terminate enzyme activity while also providing enhancement and stabilization of the fluorescent signal readout. Assay buffer is provided for viral dilution and serial NI dilution. The assay is performed using standard black microplates available from major vendors and is read on standard fluorometers, dedicated fluorescent or multimode plate readers present in many laboratories.

Comparison to NISN Protocols

The NA-Fluor™ assay reagent formulations were optimized based on comparisons to several well-established MUNANA-based assay protocols. The MUNANA substrate concentration, assay buffer formulation, and assay conditions are consistent with NISN IC_{50} determination protocols. Data generated using the NA-Fluor™ assay corresponds to data generated with established MUNANA-based protocols. This enables investigators to compare data acquired during current NI resistance surveillance screens using the NA-Fluor™ assay to data acquired using their previous MUNANA assay protocols (Figure 6).

Detection of NI-Resistant Virus in Mixed Viral Populations

The large shift in IC_{50} values between sensitive and oseltamivir-resistant virus using the NA-Fluor™ assay enables detection of mutant virus in mixed viral samples (Figure 7). This capability is critical for identifying resistant virus in clinical isolates presenting mixed populations of resistant and sensitive virus during NI susceptibility surveillance.



Protocols	Wild Type (WT) IC_{50} (nM)	Mutant (H275Y) IC_{50} (nM)	Fold Resistance to Oseltamivir
NA-Fluor™ Assay	0.42	365	869
Protocol 1	0.47	327	696
Protocol 2	0.41	271	661
Protocol 3	0.35	217	620
Protocol 4	0.36	279	775
NA-*Star*® Assay	0.2	115	575
NA-XTD™ Assay	0.19	135	711

Figure 6. NA-Fluor™ Assay Comparison of MUNANA-Based Assay Protocols for IC_{50} Determination. The NA-Fluor™ assay was run in parallel with four NISN-published MUNANA-based protocols. Oseltamivir carboxylate IC_{50} values were determined with influenza A/H1N1/Texas/36/91–sensitive and oseltamivir-resistant mutant (H275Y) strains. The NA-Fluor™ assay data corresponds with data generated using NISN-published protocols.

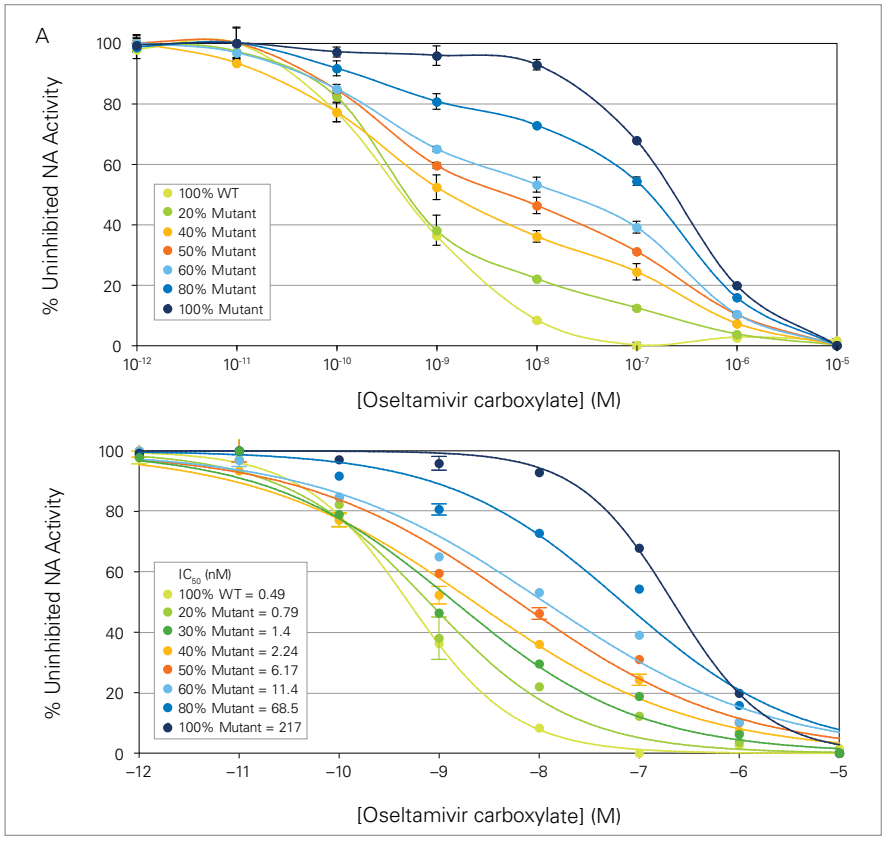


Figure 7. NA-Fluor™ Assay Detection of NI-Resistant H1N1 Virus in Mixed Populations. Sensitive and oseltamivir-resistant (H275Y) influenza A/Texas/36/91 (H1N1) strain dilutions were normalized by NA activity. **(A)** Data plotted using point-by-point graphing in Microsoft® Excel. **(B)** IC_{50} determination by curve fitting performed using GraphPad Prism® software. Subpopulations displaying drug-resistant mutations can be detected in mixtures of 50:50 sensitive and resistant virus by IC_{50} values.

Ease of Use and Flexibility

The NA-Fluor™ assay provides an easy, flexible format for screening several to hundreds of viral isolates, or for screening thousands of compounds during high-throughput lead discovery with quality data at high confidence levels. The assay has demonstrated a Z´ of 0.78–0.8, making it strongly capable for use in high-throughput screening applications. The fluorescent reaction product remains stable for hours at room temperature post-assay termination, enabling read-time flexibility and comparable data from first plate to last. Assay signal remains nearly constant and IC_{50} values (data not shown) are identical to data collected up to 4 hours at room temperature and up to 4 days at 4°C after assay termination (Figure 8).

The NA-Fluor™ assay has been optimized as an endpoint assay run at 37°C for one hour following neuraminidase inhibitor (NI) preincubation. However, the rate of MUNANA substrate turnover remains linear for more than 2 hours with viral neuraminidase, allowing the assay to be performed for as little as 20 minutes to save time or for as long as 2 hours to increase signal output. The assay can also be conducted in real time without the addition of stop solution for those investigators who want to do their own assay development or monitor rates of substrate turnover in the presence of inhibitor (Figure 9).

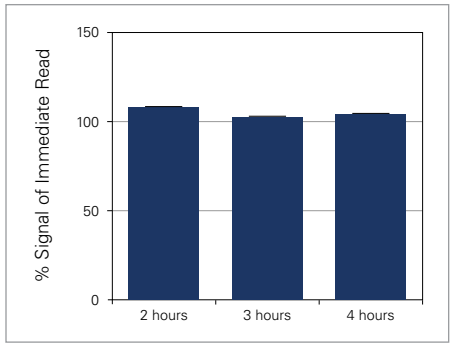


Figure 8. NA-Fluor™ Assay Signal Stability with Time. Oseltamivir IC_{50} determination assays using influenza B/Lee/40 (VR-1535™, ATCC) was performed. Plates were read immediately after addition of NA-Fluor™ Stop Solution and again at the indicated times. The assay signal intensity remains stable following prolonged plate storage. The quality of data generated with the NA-Fluor™ assay is not compromised under batch-mode assay conditions.

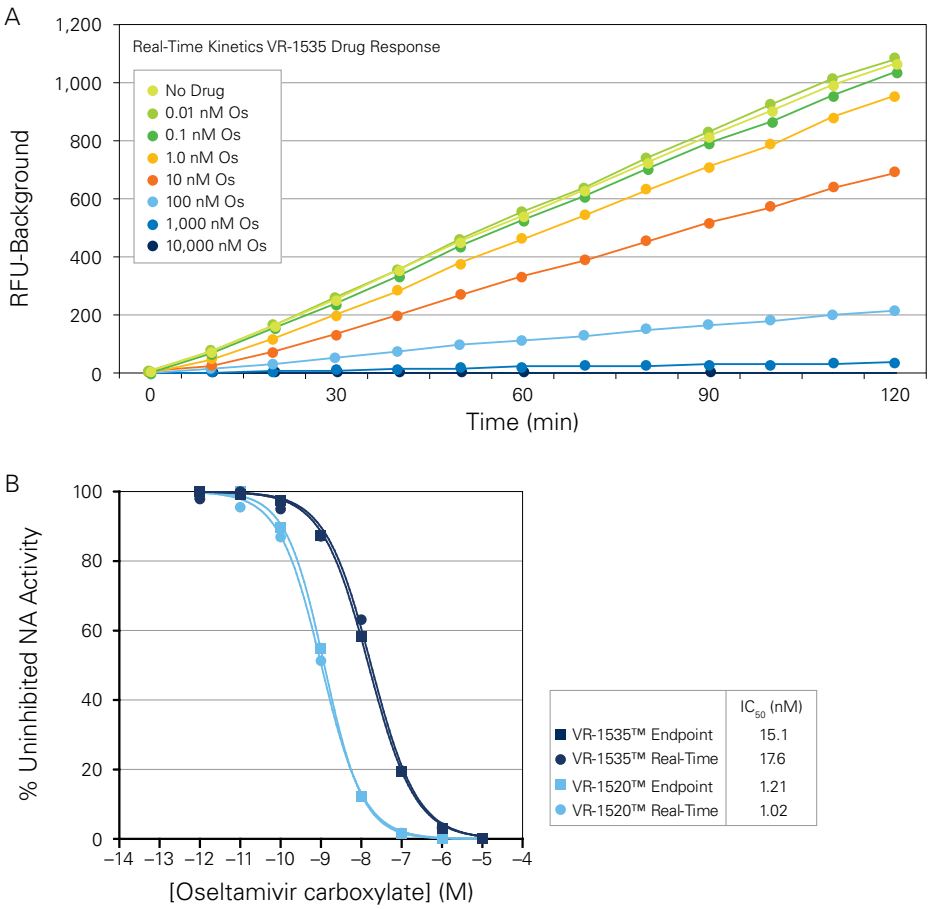


Figure 9. NA-Fluor™ Assay: Real-Time Versus EndPoint Mode Comparison. (A) Real-time kinetics of influenza strain B/Lee/40 (VR-1535™, ATCC) neuraminidase activity in the presence of varying concentrations of oseltamivir carboxylate. Real-time acquired RFUs are typically 5- to 6-fold lower than RFUs acquired after the addition of stop solution at the same time point. **(B)**Comparison of IC_{50} values for influenza strains B/Lee/40 (VR-1535™, ATCC) and A/WS/33 (H1N1) (VR-1520™, ATCC) using real-time data by slope analysis (no stop solution) or a 60 minute endpoint readout (with stop solution).

Comprehensive Assay Capability

Functional neuraminidase inhibition assays enable detection of any resistance mutation, making them extremely important for global monitoring of virus isolates for NI resistance mutations. These assays can be used in conjunction with sequence-based screening assays to identify both known and new mutations. The new NA-XTD™ and NA-Fluor™ Influenza Neuraminidase Assay Kits have been developed to offer customers optimal assay performance together with standardized reagents and protocols for performing either chemiluminescence or fluorescence-based assays, respectively. These kits provide complete solutions for performing functional neuraminidase inhibition assays, and enable additional applications, including screening and development of new neuraminidase inhibitors, virus quantitation in cell culture, and viral quantitation for vaccine research and development. Together, these assays provide highly sensitive, convenient, and versatile assay systems with standardized assay reagents and simple assay protocols.

Designed for Reliability and Consistency

The NA-Fluor™ and NA-XTD™ Influenza Neuraminidase Assay Kits are manufactured and quality control tested under rigorous quality systems that enable reliable provision of our substrates, reagents, and kits into multiple markets. There is a long development and manufacturing history of Applied Biosystems® 1,2-dioxetane enzyme substrates, chemiluminescence enhancers, substrate formulations, and complete reagent kits.

Designed for the Environment

The NA-Fluor™ Influenza Neuraminidase Assay Kit has been developed using Life Technologies' recently implemented Design for the Environment policy to ensure lowest environmental impact through the use of environmentally friendly chemistries, post-consumer waste and recyclable packaging, and low-impact shipping conditions, without compromising product performance. The NA-XTD™ Influenza Neuraminidase Assay Kit is provided with improved, environmentally-friendly accelerator formulation and kit packaging.

Worldwide Support

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

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

Table 1. NA-XTD™ and NA-Fluor™ Influenza Neuraminidase Assay Kits: Configurations and Required Instrumentation.

	NA-XTD™ Influenza Neuraminidase Assay Kit	NA-Fluor™ Influenza Neuraminidase Assay Kit
Kit Components	• NA-XTD™ Substrate • NA-XTD™ Assay Buffer • NA-XTD™ Accelerator • NA Sample Prep Buffer • NA-*Star*™ Detection Microplates: 96-well white microplates (optional) • NA-XTD™ Influenza Neuraminidase Assay Kit User Protocol	• NA-Fluor™ Substrate • NA-Fluor™ 2X Assay Buffer • NA-Fluor™ Stop Solution • NA-Fluor™ Influenza Neuraminidase Assay Kit User Protocol
Positive/Negative Control	Not included. NI-sensitive and -resistant reference influenza strains can be obtained through the CDC, other influenza reference laboratories, or through NISN (www.nisn.org). NI-sensitive influenza strains can be obtained through ATCC.	
Required Instrumentation	Microplate luminometer (or multimode instrument with luminescence capability). Onboard reagent injectors not required.	Microplate fluorometer (or multimode instrument with fluorescence capability) with approximately 360 nm excitation/460 nm emission capability. Onboard reagent injectors not required.
Data Analysis Software	Not included. Dose response (nonlinear curve fit) analysis software (for example, GraphPad Prism®) for IC_{50} determination.	

ORDERING INFORMATION

Description	Size	Part Number
NA-Fluor™ Influenza Neuraminidase Assay Kit*	Reagents sufficient for ten 96-well microplates (960 assay wells)	4457091
NA-XTD™ Influenza Neuraminidase Assay Kit	Reagents and ten solid white 96-well microplates (sufficient for 960 assay wells)	4457535
NA-XTD™ Influenza Neuraminidase Assay Reagent Set*	Reagents sufficient for ten 96-well microplates (960 assay wells)	4457534
NA-*Star*™ Detection Microplates (for use with NA-XTD™ assay only)	10 solid white 96-well microplates	4374349

*Does not include microplates.

For Research Use Only. Not for use in diagnostic procedures.

applied biosystems™ by life technologies™

Headquarters
850 Lincoln Centre Drive | Foster City, CA 94404 USA
Phone 650.638.5800 | Toll Free 800.327.3002
www.appliedbiosystems.com

International Sales
For our office locations please call the division headquarters or refer to our Web site at
www.appliedbiosystems.com/about/offices.cfm