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NA-XTD™ Influenza Neuraminidase Assay Kit

Protocol

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About This Guide

Safety information

Note: For general safety information, see this section and Appendix D, "Safety" on page 23. When a hazard symbol and hazard type appear by a chemical name or instrument hazard, see the "Safety" Appendix for the complete alert on the chemical or instrument.

Safety alert words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

SDSs

The SDSs for any chemicals supplied by Applied Biosystems are available to you free 24 hours a day. For instructions on obtaining SDSs, see "SDSs" on page 24.

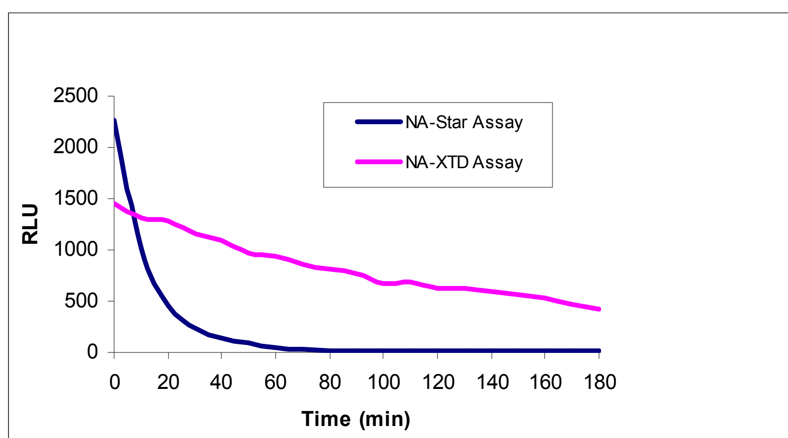
IMPORTANT! For the SDSs of chemicals not distributed by Applied Biosystems contact the chemical manufacturer.

NA-XTD™ Influenza Neuraminidase Assay Kit

Product information

Purpose of the product

The NA-XTD™ Influenza Neuraminidase Assay Kit (PN 4457535) provides the NA-XTD™ chemiluminescent substrate, assay reagents, optional microplates, and protocols to measure neuraminidase (NA) activity from influenza virus. It is the next generation of the NA-*Star*® Influenza Neuraminidase Inhibitor Resistance Detection Kit, providing longer-lasting chemiluminescent signal (see figure below) and slightly higher detection sensitivity. The assay is a direct, functional enzyme assay that detects both known and new mutations that affect neuraminidase inhibitor (NI) drug sensitivity.



The assay kit has been optimized for monitoring the effect of neuraminidase inhibitors (NI) on neuraminidase enzyme activity, a method widely used by research or public health laboratories for influenza virus NI susceptibility screening.

The NA-XTD™ Influenza Neuraminidase Assay Kit can also be used for other research applications including high-throughput screening to identify novel NIs during lead discovery, monitoring NA activity from non-viral sources, or measuring viral NA activity in culture medium as a read-out for cell-based virus growth or inhibition assays (described on on page 21).

Note: Applied Biosystems also offers a fluorescent MUNANA-based neuraminidase assay kit: NA-Fluor™ Influenza Neuraminidase Assay Kit (PN 4457091).

Kit contents and storage

Reagents for ten 96-well microplates are supplied.

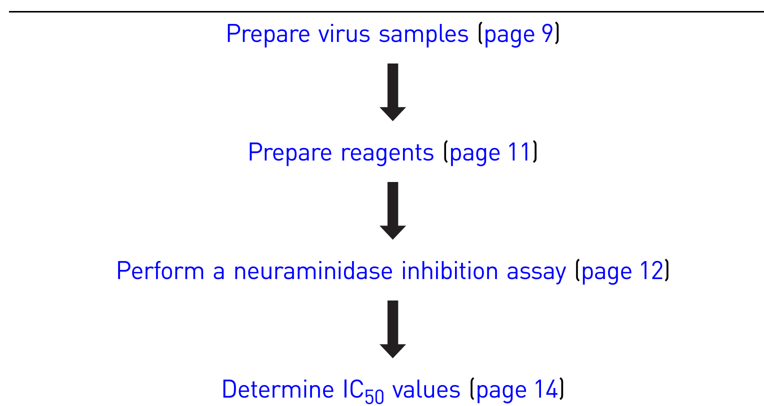
Item	Volume or Quantity	Storage Conditions
NA-XTD™ Substrate 5 mM substrate concentrate	100 µL (diluted 1:1000 with NA-XTD™ Assay Buffer before use)	2 to 8 °C
NA Sample Prep Buffer 10% Triton X-100 in NA-XTD™ Assay Buffer	5 mL	2 to 8 °C
NA-XTD™ Assay Buffer 26 mM MES (2-[N-Morpholino]ethanesulfonic acid) (pH 6.0), 4 mM $CaCl_2$	480 mL	2 to 8 °C
NA-XTD™ Accelerator Proprietary solution containing luminescence enhancer	100 mL	2 to 8 °C
NA-*Star*™ Detection Microplates (optional) Solid-white 96-well microplates	10 plates	Ambient temp

Materials and equipment required

Item	Source
Tissue culture supplies and host cell lines for virus culture preparation	Multiple laboratory supplier (MLS†)
Reference influenza virus strains (NI-resistant and NI-sensitive sub-type matched)	ATCC, NISN, or similar influenza reference laboratory NISN provides information for obtaining virus strain sets at **http://nisn.org/v_virus_reference_panel.html**
Neuraminidase inhibitors (NIs) • Oseltamivir carboxylate • Zanamivir • Other NI	See **http://nisn.org/v_neuraminidase_inhibitor_drugs.html** for contact information on obtaining NIs • Roche (Oseltamivir carboxylate) • GlaxoSmithKline (Zanamivir)
Microplate luminometer or multimode instrument with top-reading luminescence capability - does not require reagent injectors	MLS†
Lab equipment • Incubator or plate warmer: 37 °C • Freezer: –20 and –80 °C • Pipettors and tips, single-channel • Pipettors and tips, multi-channel • Reagent reservoirs, single- and multi-well	MLS†
Data analysis software – Dose-response analysis software, such as GraphPad Prism	GraphPad Software or similar supplier

† For the SDS of any chemical not distributed by Applied Biosystems, contact the chemical manufacturer. Before handling any chemicals, refer to the SDS provided by the manufacturer, and observe all relevant precautions.

Workflow



Prepare virus samples

Prepare virus stock

Prepare virus culture supernatants or egg allantoic fluid from single-passage flu virus strains using established culture methods. A comprehensive protocol for influenza virus culture and preparation of viral stocks is available at **http://www.nisn.org/documents/NISN_reference_panel_leaflet.pdf** or **http://www.atcc.org**.

If desired, you can add 1/10 volume of NA Sample Prep Buffer (which contains 10% Triton X-100 detergent) to the stock virus to obtain a 1% final concentration of Triton X-100 (for example, 100 µL NA Sample Prep Buffer + 900 µL virus stock).

Note: Triton X-100 detergent at 1% has been shown to inactivate flu virus while increasing neuraminidase (NA) activity (1). Triton X-100 detergent may increase NA activity up to 4-fold, but is not consistently observed, and seems to be most effective with more concentrated virus preparations.

Prepare virus stock dilutions

The high sensitivity and wide dynamic range of this assay allows the use of more dilute virus samples than with fluorescence-based assays, with IC_{50} values consistent over a wide range of virus concentration. A signal-to-noise ratio of 40 has been recommended as optimal for IC_{50} determinations (2, 4), with a minimum recommended signal-to-noise ratio of 10.

In general, the following virus dilutions provide an appropriate virus concentration for IC_{50} determination:

- A 1:5 dilution provides consistent results with low-to-medium virus yields, with negligible quenching effect from phenol red.
- A 1:50 to 1:100 dilution can be used for virus cultures with higher yield.

Note: Samples with very low virus concentration can result in unreliable IC_{50} values.

(Optional) Titer the virus stock to determine the optimum dilution

If desired, you can assay serial dilutions of the virus stock to determine the optimal virus dilution and minimize the amount of virus stock used for the assay. The optimal dilution depends on the level of NA activity in a particular virus strain and on the virus yield.

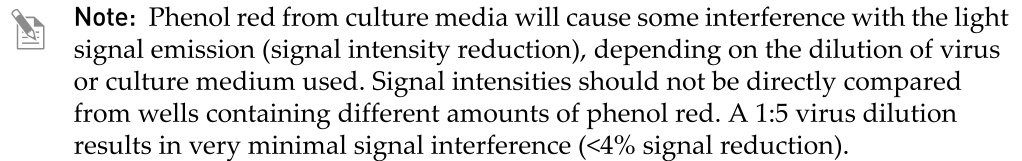
- Prepare serial 1:2 dilutions of virus stock (containing NA Sample Prep Buffer if added in "Prepare virus stock" on page 9) with NA-XTD™ Assay Buffer.
- Follow the neuraminidase inhibition assay protocol on page 12, but replace the NI volume in step 1 with 25 µL of NA-XTD™ Assay Buffer.
- Determine the signal-to-noise ratio for each dilution (using signal from either culture medium from uninfected cells or assay control (no virus, no neuraminidase inhibitor (NI) as the noise).

Prepare dilutions

1. Determine the total volume of diluted virus sample needed:

   25 µL/well × 11 × number replicates + appropriate extra volume for pipetting reservoir/error

2. Dilute the virus stock at the desired dilution in NA-XTD™ Assay Buffer.



Note: Phenol red from culture media will cause some interference with the light signal emission (signal intensity reduction), depending on the dilution of virus or culture medium used. Signal intensities should not be directly compared from wells containing different amounts of phenol red. A 1:5 virus dilution results in very minimal signal interference (<4% signal reduction).

Phenol Red, g/L	Media Dilution	% Signal Reduction
0.01	undiluted	31%
0.005	1:2	8%
0.0025	1:4	4%
0.00125	1:8	2%
0.000625	1:16	<1%
0.0003125	1:32	<1%
0.00015625	1:64	<1%

Prepare reagents

Prepare neuraminidase inhibitors (NI)

Prepare master stock

Prepare a 25 mM master stock of each inhibitor:

- Oseltamivir carboxylate (D-tartrate salt, MW = 386.4) is soluble in water. Dissolve 19.3 mg in 2 mL distilled water.
- Zanamivir (MW = 332.3) is soluble in water up to 18 mg/mL. Dissolve 17.5 mg in 2 mL distilled water.
- Other NIs: Prepare and dissolve according to manufacturer recommendations.

Aliquot and store according to manufacturer recommendations. Avoid freezing-thawing.

Prepare working stock

1. Dilute the 25 mM master stock 1:50 (50 µL 25 mM master stock + 2450 µL H_2O) to make 500 µM working stock. Aliquot and store the working stock according to manufacturer recommendations; use working stock aliquots upon thaw and discard unused portions.

2. Prepare serial dilutions of NIs in NA-XTD™ Assay Buffer. Total volume of each NI dilution required is 25 µL/well (200 µL/plate plus extra).

   Example: A suggested dilution scheme is shown below and provides a wide range of NI dilutions resulting in complete inhibition of the A/H1N1/TX/36/91/H275Y virus.

   Note: For prior NA-*Star*® assay users, the drug dilution scheme, starting concentration, and volumes have been adjusted from the NA-*Star* assay protocol to ensure complete inhibition of resistant viruses.

Dilution	Combine	NI concentration before assay (3X)	NI concentration in final assay
1 (1:25)	30 µL WS† NI + 720 µL AB‡	20,000 nM	6600 nM
2 (1:5)	100 µL Dil 1 + 400 µL AB	4000 nM	1320 nM
3 (1:5)	100 µL Dil 2 + 400 µL AB	800 nM	264 nM
4 (1:5)	100 µL Dil 3 + 400 µL AB	160 nM	52.8 nM
5 (1:5)	100 µL Dil 4 + 400 µL AB	32 nM	10.56 nM
6 (1:5)	100 µL Dil 5 + 400 µL AB	6.4 nM	2.11 nM
7 (1:5)	100 µL Dil 6 + 400 µL AB	1.28 nM	0.422 nM
8 (1:5)	100 µL Dil 7 + 400 µL AB	0.256 nM	0.084 nM
9 (1:5)	100 µL Dil 8 + 400 µL AB	0.0512 nM	0.017 nM
10 (1:5)	100 µL Dil 9 + 400 µL AB	0.01 nM	0.003 nM
11	400 µL AB	0.0 nM	0.0 nM

† Working Stock (WS) = 500 µM NI
‡ NA-XTD™ Assay Buffer (AB)

Note: For compound screening for neuraminidase inhibition, prepare compounds at 3X the desired final concentration in NA-XTD™ Assay Buffer. A final concentration of 5% DMSO can be tolerated in the assay, although all wells should contain the same concentration of DMSO, including control wells.

Prepare NA-XTD™ Substrate

Note: Before opening the NA-XTD™ Substrate, spin the tube briefly in microcentrifuge to ensure that all volume is in bottom of tube.

1. Determine the volume of substrate needed. Prepare sufficient volume for immediate use (25 µL/well plus sufficient excess for reagent reservoir). Do not save unused diluted NA-XTD™ Substrate.

   Example: For one 96-well plate, prepare a total of 4 mL of diluted NA-XTD™ Substrate (4 µL NA-XTD™ Substrate + 4 mL NA-XTD™ Assay Buffer). This volume provides 2400 µL total volume required for the assay wells with ~1.5 mL excess volume.

2. Dilute the NA-XTD™ Substrate 1:1000 in NA-XTD™ Assay Buffer (at room temperature) immediately before use.

Perform a neuraminidase inhibition assay

Reference viruses

The following subtype-matched reference viruses are recommended in each assay run (use reference strains appropriate for virus isolates and neuraminidase inhibitors (NIs) you are screening):

- An NI-sensitive strain
- Corresponding strains resistant to zanamivir, oseltamivir carboxylate, or other NI you are using

Note: If you run multiple plates, reference viruses are not required on all plates.

Plate layout

An example of a plate layout is shown in Table 1. Use column(s):

- 1 to 11 to determine IC_{50} value.
- 11 to provide a readout of viral neuraminidase (NA) activity in the absence of NI (untreated).
- 12 no virus assay control to provide an assay background or “noise” readout (signal resulting from combination of reagent background and presence of any non-viral NA activity present).

Column contents:

- 1 through 11 contain the diluted virus sample
- 12 contains assay control (uninfected MDCK cell culture supernatant at the same dilution as the virus stock, or assay buffer only).

Table 1 Example plate layout

	Final NI conc (nM)											
	6600	1320	264	52.8	10.56	2.11	0.422	0.084	0.017	0.003	0	0
	1	2	3	4	5	6	7	8	9	10	11	12
A	Virus 1†											No Virus Assay Control
B												
C	Virus 2											
D												
E	Virus 3											
F												
G	Virus 4											
H												

† Include reference viruses as appropriate. See "Reference viruses" on page 12 for recommendations.

Note: Other plate layouts can be used, depending on the desired NI dilution series, number of virus samples per plate, and number of replicates.

Perform the neuraminidase inhibition assay

Refer to the plate layout in Table 1 to prepare the assay plate:

1. Add 25 µL of NI dilution to wells in columns 1 through 10.
2. Add 25 µL of NA-XTD™ Assay Buffer to wells in column 11 and 12.
3. Add samples:
   a. 25 µL of diluted virus sample per well in duplicate (columns 1 through 11).
   b. 25 µL of No Virus Assay Control (uninfected MDCK cell culture supernatant or NA-XTD™ Assay Buffer) (column 12).

   Total well volume is 50 µL before addition of diluted NA-XTD™ Substrate.
4. Place the lid on the plate, then incubate for 20 minutes at 37 °C.
5. Add 25 µL of diluted NA-XTD™ Substrate to each well.
6. Place the lid on the plate, then incubate for 30 minutes at ambient temperature.
7. Add 60 µL of NA-XTD™ Accelerator to each well.
8. Read the plate using a 1 sec/well read time.
   Plates can be read immediately, or up to 2 hours after adding NA-XTD™ Accelerator.

   Note: After NA-XTD™ Accelerator addition, the light signal reaches maximum intensity within minutes and slowly decays with a half-life of light emission of approximately 2 hours. IC_{50} values determined up to 3 hours after adding NA-XTD™ Accelerator are identical to IC_{50} values determined immediately.

Determine IC_{50} values

Determine IC_{50} values from dose-response data using sigmoidal curve-fitting or point-to-point plotting. You can use any software that can perform sigmoidal curve-fitting or point-to-point plotting. See the NISN web site (**http://www.nisn.org**) for detailed information on determining, interpreting, and troubleshooting IC_{50} results, and for criteria for defining NI-resistant virus strains.

Guidelines for GraphPad Prism Software

This section provides some guidelines for analyzing data if you use GraphPad Prism Software for sigmoidal curve-fitting of dose response data (for more information, see the *GraphPad Prism Step-by-Step Examples Guide* or contact the manufacturer).

1. Select the graph type (typically XY, points only graph with Y error bars) and number of replicates.
2. Enter data:
   a. X Values: enter NI concentrations in molar concentration in exponential notation. Enter a concentration that is 100 to 1000-fold lower than lowest NI dilution to use as the "0" NI value.
   b. Y Values: enter response data in appropriate sets of Y columns. Raw signal intensity (dose response) data is exported into software.
3. Data manipulation/analyze:
   a. Log Transform X Values: in Analyze:Transforms dialog box, select X=Log(X)
   b. Normalize Y Values: in the Analyze:Normalize dialog box, define 0% as the smallest values and 100% as the highest values (default), present results as Percentages and Create a new graph.
4. Curve-fitting:
   a. With the graph generated above displayed, click Analyze:Nonlinear regression, select Sigmoidal dose-response (variable slope), (same as "four-parameter logistic" in other graphing programs).
   b. (Optional) Constrain curve-fit parameters by clicking the Constraints tab and set BOTTOM parameter to 0.0 and TOP parameter to 100.0.

      Note: Constraining the parameters may alter the determined IC_{50} value.
5. Graph adjustments:
   a. Values/data from the Results page can be copied/pasted onto the Graph page to display curve-fit parameters on graph.
   b. X-axis and Y-axis adjustments can be made to alter range, to segment the X-axis, change concentration display, etc.

Troubleshoot

Table 2 Neuraminidase inhibition assay troubleshooting

Observation	Possible cause	Recommended action
Low signal	Incorrect plates used	Use the solid, white microplates provided with kit, or other solid, white plates. Do not use black microplates (which will absorb luminescent signal).
	Instrument incorrectly set up	Set for luminescence measurement.
	High concentration of phenol red	Use more diluted virus sample.
		If undiluted virus stock must be used, use the NA-Fluor™ Influenza Neuraminidase Assay Kit (PN 4457091) which has no phenol red interference.
	Reagent problem	Confirm reagent activity by running the assay using as sample purified bacterial neuraminidase (New England Biolabs #P0720L (C. perfringens) or #P0728S (S. typhimurium) or equivalent). Prepare serial dilutions of purified bacterial neuraminidase (NA) in NA-XTD™ Assay Buffer containing 100 mg/mL BSA. Neuraminidase quantitation in the range of sub-pM to nM is typically achieved, depending on the specific activity of the enzyme preparation.
	Virus yield is poor or NA activity is extremely low	Use a lower dilution or undiluted virus. High concentration of phenol red will reduce signal output, but IC_{50} data may provide acceptable curve fit.
Higher than expected signal in some wells	Well-to-well signal cross-talk	Do not use clear microplates (which will cause extensive well-to-well signal cross-talk.)
High background	Incorrect plates used	Use the solid, white microplates provided with kit, or other solid, white plates.
	Plate is emitting phosphorescence	Allow the plate to sit in the reader for 5 minutes before reading to allow phosphorescence from the plate to decay.

Ordering and Support Information

How to order

To place an order online, go to **www.appliedbiosystems.com**.

To place an order from the U.S. or Canada, dial 1-800-345-5224, then follow the voice instructions.

To place an order outside the U.S., go to www.appliedbiosystems.com to locate your nearest Applied Biosystems office.

Description	Quantity	Part Number
NA-XTD™ Influenza Neuraminidase Assay Reagent Set	Reagents sufficient for 960 assays (100 µL) or ten 96-well assay microplates	4457534
NA-XTD™ Influenza Neuraminidase Assay Kit	• Reagents sufficient for 960 assays (100 µL) or ten 96-well assay microplates • 10 microplates	4457535

Support

For the latest services and support information for all locations, go to:

www.appliedbiosystems.com

At the Applied Biosystems web site, you can:

- Access worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities (for Product/Area, select **Chemiluninescence/Tropix**).
- Search through frequently asked questions (FAQs).
- Submit a question directly to Technical Support.
- Order Applied Biosystems user documents, SDSs, certificates of analysis, and other related documents.
- Download PDF documents.
- Obtain information about customer training.
- Download software updates and patches.

Background Information

Neuraminidase inhibitor (NI) resistance monitoring

Viral neuraminidase (NA) is the target of key antiviral drugs effective in treating the influenza strains that are currently considered world-health threats. Influenza virus neuraminidase functions by allowing release of newly formed viral particles from a host cell by cleaving terminal N-acetyl neuraminic acid (sialic acid) which serves as the attachment site between the viral hemagglutinin (HA) protein and glycoconjugates on the host cell surface. Neuraminidase inhibitor (NI) drugs, including oseltamivir (Tamiflu®) and zanamivir (Relenza®), as well as drugs awaiting approval,act by binding competitively to the active site of the neuraminidase, thus inhibiting release and spread of the virus. Mutations in viral neuraminidase have been identified which render the virus resistant to NIs, resulting in the need to survey influenza-strain susceptibility to this class of antiviral drugs. Viral susceptibility or resistance can be monitored by measuring the drug inhibitory effect on NA enzyme activity.

NI resistance monitoring is critical to track treatment efficacy and global spread of resistant viral strains for seasonal, avian, animal and pandemic strains. Increasingly, since the introduction of neuraminidase inhibitor therapeutics in 1999, mutations conferring resistance to neuraminidase inhibitors are arising and spreading, with seasonal A/H1N1 isolates predominantly resistant to oseltamivir globally**.** Functional neuraminidase inhibition assays enable detection of any resistance mutation, and are therefore extremely important in conjunction with sequence-based screening assays for global monitoring of virus isolates for NI resistance mutations, including known and new mutations.

Chemiluminescence technology

Neuraminidase assays performed with chemiluminescent 1,2-dioxetane substrates, including NA-*Star*® and NA-XTD™ Substrates, provide 5-to-50-fold higher sensitivity by signal-to-noise ratio than assays performed with the fluorescent MUNANA substrate. In addition, chemiluminescent assays provide linear results over 3 to 4 orders of magnitude of neuraminidase concentration compared to 2 to 3 orders of magnitude achieved with the fluorescent MUNANA-based assay (2).

The NA-XTD™ chemiluminescent substrate (sodium (3-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) is nearly identical to the NA-*Star*® Substrate, with a single structural difference that provides a much longer-lasting chemiluminescent signal with slightly higher detection sensitivity. The NA-XTD™ Substrate provides a chemiluminescent signal half-life of approximately 2 hours, eliminating the need for reagent injectors and enabling more convenient batch-mode processing of assay plates.

The first generation NA-*Star*® assay has been widely used for global monitoring of NI sensitivity, with influenza virus types A/H1N1 and A/H3N2 and B, including human, avian, porcine and equine viruses (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20), including identification of recent A/H1N1 pandemic virus strains resistant to oseltamivir (21). In addition, the NA-*Star* assay has been used for identification of new NI compounds (22), NI characterization (23, 24, 25, 26), clinical and animal studies of NI-resistance development, virus transmission, and drug delivery (27, 28, 29), NA quantitation of virus-like particles (30), and cell-based virus quantitation (31).

IC_{50} results with the chemiluminescent assays typically show good correlation with fluorescent assay results, with slight differences in IC_{50} values (2, 3).

Cell-Based Virus Growth Assay

Overview

The NA-XTD™ Influenza Neuraminidase Assay Kit can be used to measure influenza virus growth or growth inhibition in cell-based assays by measuring the amount of neuraminidase (NA) activity in a 96-well microplate cell culture or a sample of cell culture medium. Applications include measuring viral growth inhibition in the presence of inhibitory antibodies or compounds, described as AVINA (**A**ccelerated **V**iral **I**nhibition with **NA** as read-out assay (31)).

Prepare virus culture

Select microplates

If you will perform the assay directly in a 96-well microplate used for viral culture, eliminating need for separate assay plate, use white tissue culture-treated microplates with a clear bottom. Do not use clear 96-well tissue culture-treated microplates if you will perform the assay in the microplate used for viral culture.

If you will not perform microscopic observation of cells/virus cultures, use solid white tissue culture-treated microplates.

If you will perform the assay in a separate assay microplate, virus/cell cultures can be conducted in any desired tissue culture-treated plates.

Note: Performing the assay using the entire well contents (~100 µL) of culture volume reduces detection sensitivity due to a high concentration of phenol red. To minimize phenol red interference and improve sensitivity, you can:

- Transfer 25 to 50 µL of culture volume to another plate, then perform the assay. This method leaves volume in the culture well for temporal monitoring of virus growth/inhibition, or for other assays such as ELISA quantitation of NP protein.
- Remove some of the culture medium from the culture plate, then perform the assay in the culture plate on a smaller volume of the culture well.

Note: The use of clear-bottom microplates can result in well-to-well signal cross-talk, particularly if high signal wells are adjacent to low signal wells. To minimize cross-talk, design the plate layout to avoid placing the highest signal assay wells adjacent to the lowest signal assay wells. In addition, when using clear-bottom microplates, use a white adhesive plate backing sheet (Perkin-Elmer, PN 6005199) to maximize light signal reaching detector.

Prepare cultures

1. Seed virus host cells (for example, MDCK cells) at the desired density, then culture overnight.
2. Infect virus host cells with the desired virus dilution in virus infection medium.
   a. If you are performing an antibody inhibition experiment, add antibody dilutions to the virus host cells.
   b. If you are performing a drug inhibition experiment, add drug dilutions either with the virus or after an infection incubation.
3. Incubate virus cultures for the desired time and assay at the desired time points.

Perform the assay

To perform the assay in the 96-well virus culture plate:

1. (Optional) To optimize sensitivity, remove partial volume of the culture medium from culture wells, leaving 50 µL of culture medium remaining in well for the assay.
2. Add 25 µL of 1:1000 NA-XTD™ substrate diluted in NA-XTD™ Assay Buffer, then incubate for 30 minutes at ambient temperature.
3. If using a clear-bottom plate, apply adhesive white backing sheet to bottom of plate.
4. Add 60 µL of NA-XTD™ Accelerator.
5. Read the plate for 1 sec/well.

To perform the assay on a sample of virus culture medium taken from a culture plate or alternate size growth vessel:

1. Remove a 25 to 50 µL aliquot of virus culture medium to a separate NA-Star Detection Microplate or other 96-well white assay plate.
2. Add 25 µL of 1:1000 NA-XTD™ substrate diluted in NA-XTD™ Assay Buffer, then incubate for 30 minutes at ambient temperature.
3. Add 60 µL of NA-XTD™ Accelerator.
4. Read the plate for 1 sec/well.

Safety

This appendix covers:

- Chemical safety 23
  - General chemical safety 23
  - SDSs 24
  - Chemical waste safety 25
  - Biological hazard safety 26

Chemical safety

General chemical safety

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL HAZARD. All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About SDSs" on page 24.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

SDSs

About SDSs

Chemical manufacturers supply current Safety Data Sheets (SDSs) with shipments of hazardous chemicals to new customers. They also provide SDSs with the first shipment of a hazardous chemical to a customer after an SDS has been updated. SDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new SDS packaged with a hazardous chemical, be sure to replace the appropriate SDS in your files.

Obtaining SDSs

The SDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain SDSs:

1. Go to **www.appliedbiosystems.com**, click **Support**, then select **SDS**.
2. In the Keyword Search field, enter the chemical name, product name, SDS part number, or other information that appears in the SDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

Note: For the SDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical waste safety

Chemical waste hazards

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets and local regulations for handling and disposal.

WARNING! CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying a waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

General biohazard

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/ nara/cfr/waisidx_01/29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

www.cdc.gov

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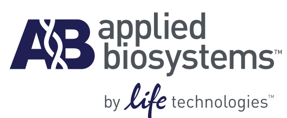

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