

TaqMan® NDV Reagents

Do not store in a frost-free freezer.

Catalog # (P/N): 4406874

Materials Provided: Reagents for 100, 25 µL RT-PCRs are supplied. Store the reagents in a non-frost-free freezer.

Component	Amount	Storage
2X RT-PCR Buffer	1.375 mL	-20°C
25X RT-PCR Enzyme Mix	110 µL	-20°C
25X NDV Primer Probe Mix	110 µL	-20°C
Nuclease-free Water	1.75 mL	-20°C, 4°C, or room temp

TaqMan® Probe Reporter/Quencher:

Target	Reporter	Quencher
NDV	FAM™ dye	BHQ®-1 dye
Xeno™ RNA Control	CF560 dye*	BHQ-1 dye

*Cal Fluor® Orange 560 (CF560) dye has an absorbance maximum of 538 nm and an emission maximum of 559 nm. When using an Applied Biosystems Real-Time PCR System, it is best to calibrate with Cal Fluor Orange 560 dye. If that is not possible, use the VIC™ dye detector for Cal Fluor Orange 560 dye.

Safety Information:

Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING! CHEMICAL HAZARD. 2X RT-PCR Buffer causes eye, skin, and respiratory tract irritation. May be harmful if swallowed, inhaled, or absorbed through skin. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.

WARNING! CHEMICAL HAZARD. 25X RT-PCR Enzyme Mix may cause eye, skin, and respiratory tract irritation. May be harmful if swallowed. Avoid breathing vapor. Use with adequate ventilation. Avoid contact with eyes and skin.

USER INFORMATION

General Information:

TaqMan® NDV Reagents enable one-step, real-time, RT-PCR targeting Newcastle Disease Virus RNA and Xeno RNA Control. The RT-PCR tolerates a wide range of input RNA amounts, but we typically do not exceed 100 ng of input RNA.

RNA isolation

- Add Xeno RNA Control to the lysis solution used for RNA isolation, to serve as a positive control both for recovery of RNA and for the RT-PCR. Add 5,000–10,000 copies of Xeno RNA Control per isolation (0.5–1.0 µL at 10,000 copies/µL).
- When isolating RNA from cell-free sample sources such as serum, add carrier RNA to the lysis solution to maximize RNA recovery. Include carrier RNA even if you have added Xeno RNA Control to the lysis solution.
- It is important to use RNA that is free of RT-PCR inhibitors in the procedure.
- To isolate RNA for use in this procedure, we recommend using the Ambion® MagMAX™ RNA Isolation Kit appropriate for your sample type. Search for "MagMAX" at www.appliedbiosystems.com.

Applications:

Reaction setup

1. Program the real-time PCR instrument according to the manufacturer's recommendation.

ROX passive reference dye is included in the RT-PCR Buffer.

2. Assemble RT-PCR master mix(es), for 25 µL reactions, on ice. Include 5–10% extra to ensure that you will have enough master mix for all the samples.

Component	Amount
2X RT-PCR Buffer	12.5 µL
25X NDV Primer Probe Mix	1 µL
25X RT-PCR Enzyme Mix	1 µL
Total volume master mix per reaction	14.5 µL

3. Distribute RT-PCR master mix to the wells of a PCR plate or to PCR tubes.
4. Add sample or Nuclease-free Water for a final reaction volume of 25 µL.
5. Run the thermal cycler program.

QUALITY CONTROL

Functional Testing:	Each lot of supplied reagent is tested for detection of RNA target using the instructions in this protocol. Relevant kit components are tested in the following nuclease assays.
RNase Activity:	A sample is incubated with labeled RNA, followed by PAGE analysis.
Nonspecific Endonuclease Activity:	A sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.
Exonuclease Activity:	A sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

OTHER INFORMATION

Material Safety Data Sheets:	Material Safety Data Sheets (MSDSs) for any chemical product supplied by Applied Biosystems or Ambion are available 24 hours a day. At www.appliedbiosystems.com , select Support, then MSDS. Search by chemical name, product name, product part number, or MSDS part number. Right-click to print or download the MSDS of interest. At www.ambion.com , go to the web catalog page for the product of interest. Click MSDS, then right-click to print or download. Or, e-mail (MSDS_Inquiry_CCRM@appliedbiosystems.com) or telephone (650-554-2756; USA) your request, specifying the catalog or part number(s) and the name of the product(s). We will e-mail the associated MSDSs unless you request fax or postal delivery. Requests for postal delivery require 1-2 weeks for processing.
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