VetMAX™ NDV Kit

TaqMan® real-time RT-PCR for the detection of paramyxovirus type 1

Catalog Number NDV

Doc. Part No. 100020401 **Pub. No.** MAN0008826 **Rev.** B.0

Technology	Species	Samples	Test type
Real-time RT-PCR (RNA) • Duplex assay • Xeno™ RNA Control	Poultry	Tracheal, cloacal swabs Feces Tissues	Individual

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

WARNING! POTENTIAL BIOHAZARD. Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Product information

Name, intended use, and principle of the procedure

The **Applied Biosystems™ VetMAX™ NDV Kit** (Cat. No. NDV) enables real-time reverse transcription PCR (RT-PCR) detection of paramyxovirus type 1 extracted from swabs, feces, or tissues.

NDV is an enveloped, single-stranded RNA type 1 paramyxovirus responsible for Newcastle Disease, an infectious and contagious disease which affects domestic and wild birds.

The VetMAX™ NDV Kit provides assays and reagents required for single-well, real-time RT-PCR in which RNA is transcribed to cDNA, and NDV and Xeno™ RNA targets are amplified and detected using fluorescent TaqMan® probes (hydrolysis probe chemistry). It contains:

- 25X NDV Primer Probe Mix: primers and TaqMan® probes for optimized duplex real-time PCR amplification of NDV target and Xeno™ RNA target.
- 2X qRT-PCR Buffer: RT-PCR buffer.
- 25X qRT-PCR Enzyme Mix: RT-PCR enzyme.
- 25X NDV Control RNA: serves as an external positive control for the real-time RT-PCR components and it is also used to set the cycle threshold (C_t) for evaluating test results.
- Xeno™ RNA Control: the internal positive control is added to each sample and control at the lysis step of the RNA extraction procedure. It serves as an internal positive control for the RNA purification process and monitors for the presence of RT-PCR inhibitors.

Kit contents and storage conditions

Reagents for 100 25-µL real-time RT-PCR tests are supplied.

Component	Volume	Storage
25X NDV Primer Probe Mix	110 µL	–30°C to –10°C
2X qRT-PCR Buffer	1375 µL	–30°C to –10°C
25X qRT-PCR Enzyme Mix	110 µL	–30°C to –10°C
Nuclease-free Water	1750 µL	–30°C to +25°C
25X NDV Control RNA	15 µL	–25°C to –15°C
Xeno™ RNA Control (10,000 copies/µL)	110 µL	–25°C to –15°C
Nucleic Acid Dilution Solution	500 µL	–30°C to –10°C

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Real-time PCR thermal cycler capable of detecting: • FAM™ (maximum emission: λ515 nm) • CAL Fluor™ Orange 560 (maximum emission: λ561 nm)	MLS
96-well plate, PCR strips (8- or 12-wells), microtubes or capillaries compatible with thermal cycler used	MLS
Suitable plate covers or caps	MLS
Nuclease-free pipettes and filtered pipette tips	MLS
Nuclease-free reagent tubes for preparing master mix	MLS
2 ice buckets or refrigerated racks: • One for the PCR setup area where the RT-PCR master mix is prepared • One for the area where RNA may be present	MLS
1X TE buffer	MLS
DNase/RNase-free water	MLS

Isolate RNA from samples

RNA extraction from initial samples is required prior to real-time PCR analysis.

Step or process	Recommendation
Preparation of mock-purified samples for use as extraction control	Prepare mock-purified samples, using nuclease-free water as the starting material. Process with the same RNA isolation method that is used for test samples.
Proposed RNA isolation methods	MagMAX™-96 Viral RNA Isolation Kit (Cat. Nos. AM1836, AMB18365) or an equivalent RNA isolation method.
Required modifications to the RNA isolation method	• Add 1 µL of undiluted Xeno™ RNA Control per isolation to the lysis solution used for RNA isolation. • Add carrier RNA to the lysis solution according to the manufacturer recommendations. Carrier RNA is provided in the MagMAX™-96 Viral RNA Isolation Kit (Cat. Nos. AM1836, AMB18365)

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Perform real-time RT-PCR

1 Determine the quantity of reactions and thaw the reagents.

a. For each real-time RT-PCR run, include the following reactions:

- Positive control; use 10.5 µL of diluted NDV Positive Control.
- Extraction control; use 10.5 µL of mock-purified samples.
- No-template control; use nuclease-free water in place of sample RNA.

b. Thaw PCR master mix reagents in one ice bucket or refrigerated rack and controls and samples in a separate ice bucket or refrigerated rack, gently vortex each tube to mix the contents thoroughly, then briefly centrifuge to collect the solution at the bottom of the tube. Keep the reagents on ice or on a refrigerated rack.

2 Prepare the diluted NDV Positive Control.

Combine the following components and place the mixture on ice between 2ºC and 8ºC for immediate use, or below –16ºC for later use.

Component	Volume
Nucleic Acid Dilution Solution	9.5 µL
25X NDV Control RNA	1.0 µL
Total volume of diluted NDV Positive Control	10.5 µL

3 Prepare the real-time RT-PCR master mix on ice.

Combine the following components for the number of reactions required plus 10% overage.

Component	Volume per reaction
2X qRT-PCR Buffer	12.5 µL
25X qRT-PCR Enzyme Mix	1.0 µL
25X NDV Primer Probe Mix	1.0 µL
Total volume of real-time RT-PCR master mix	14.5 µL

4 Set up the real-time RT-PCR reactions.

a. Dispense 14.5 µL of real-time RT-PCR master mix to the appropriate PCR plate well, PCR strips, or capillaries.

b. Add the appropriate component for the sample type, according to the following table:

Sample type	Component	Volume per reaction
Test sample	Sample RNA	10.5 µL
Positive control	Diluted NDV Positive Control	10.5 µL
Extraction control	Mock-purified sample	10.5 µL
No-template control	Nuclease-free water	10.5 µL

c. Seal each reaction vessel.

5 Set up and run the real-time RT-PCR instrument.

a. Following the manufacturer's instructions, set up the following parameters for the real-time RT-PCR run.

- Reaction volume: 25 µL
- ROX™ passive reference dye: Included in 2X qRT-PCR Buffer (required if the thermal cycler is capable of ROX™ detection)

b. Set up and assign TaqMan® probe reporter dyes and quenchers for each well, tube, or capillary used in the analysis:

Target	Reporter	Quencher
NDV	FAM™ dye	BHQ™-1 dye
Xeno™ RNA Control	CAL Fluor™ Orange 560 dye	BHQ™-1 dye

Note: Use CAL Fluor™ Orange 560 dye to calibrate the thermal cycler, if possible. Otherwise use equivalent dye detectors such as VIC™.

c. Run the thermal cycler program and collect real-time amplification data during the elongation step (45 seconds at 60ºC). Use the following thermal cycler settings:

Stage	Repetitions	Temperature	Time
1	1×	48ºC	10 minutes
2	1×	95ºC	10 minutes
3	40×	95ºC	15 seconds
		60ºC	45 seconds

Data analysis

Refer to the recommendations of the thermal cycler manufacturer for raw data analysis.

1. Set thresholds separately for each real-time RT-PCR target.
2. Interpret the results for each control and samples according to the obtained C_t values as indicated in the following section.

Validation

The run is validated if the following criteria are met.

Control reaction	C_t value for NDV RNA	C_t Value for Xeno™ RNA Control
Positive control	24–27	>40 (no signal detected)
Extraction control	>40 (no signal detected)	29–34
No-template control	>40 (no signal detected)	>40 (no signal detected)

Interpretation

C_t value for NDV RNA	C_t Value for Xeno™ RNA Control	Interpretation
<40	<40	Sample positive for NDV
>40 (no signal detected)	< C_t Extraction control ± 3C_t	Sample negative for NDV
>40 (no signal detected)	>40 (no signal detected)	Not validated[1]

[1] See "Troubleshooting".

Troubleshooting

Observation	Possible cause	Recommended action
Test samples Xeno™ RNA Control—no signal NDV RNA—high signal	The Xeno™ RNA Control primers and probes are present at limiting concentrations in the RT-PCR reactions. High levels of NDV RNA in a sample can reduce amplification of Xeno™ RNA Control.	No or low signal from the Xeno™ RNA Control is expected in a reaction that has a strong signal for NDV RNA.
Test samples NDV RNA—no signal Xeno™ RNA Control—no signal	Xeno™ RNA Control was omitted	Verify that Xeno™ RNA Control was added to the lysis solution during RNA extraction.
	Poor RNA recovery	Check the recovery of RNA carrier used in RNA extraction.
	The RNA samples contain inhibitors of RT-PCR	Reduce the quantity of sample added to the RT-PCR reactions. For example: • Add 1–2 µL of sample (add nuclease-free water to bring the reaction to the proper volume) • Dilute the RNA sample 1:10 in Nucleic Acid Dilution Solution before adding it to the reaction. • The C_t values for Xeno™ RNA Control and NDV amplifications are expected to decrease proportionally to the decrease in sample quantity (~2-3C_t).
	Poor RNA recovery Or The RNA samples contain inhibitors of RT-PCR	Compare the results of amplifying sample RNA using RT-PCR master mix with and without 1 µL of Xeno™ RNA Control: • If the reactions amplified using the RT-PCR master mix with addition of Xeno™ RNA Control return a signal, but the reactions amplified using the RT-PCR master mix without addition of Xeno™ RNA Control return no signal, Xeno™ RNA Control was not recovered. • If Xeno™ RNA Control signal is not detected in either sample, the RNA sample contains inhibitors of real-time RT-PCR.

Observation	Possible cause	Recommended action
Positive control reaction NDV Positive Control—no signal Xeno™ RNA Control—no signal	The NDV Positive Control was improperly handled, resulting in RNA degradation.	Use appropriate precautions against RNase contamination when handling the control RNAs. For example, wear clean gloves and use nuclease-free barrier pipette tips.
	The 25X qRT-PCR Enzyme Mix was stored or handled improperly, and it lost activity.	Repeat the RT-PCR with fresh reagents.
	The thermal cycler was not properly set up.	Check the thermal cycler settings. See "Set up and run the real-time PCR instrument." on page 5.
	The RT-PCR master mix was prepared incorrectly.	Repeat the test with correctly prepared RT-PCR master mix.
No-template control reaction NDV RNA—signal detected or Xeno™ RNA Control—signal detected	Contamination during the PCR.	• Repeat the real-time RT-PCR with fresh reagents and freshly decontaminated pipettes. • Set up the real-time RT-PCR in an area separate from areas used for RNA isolation and PCR product analysis.
Extraction control reaction NDV RNA—signal detected	Contamination during RNA extraction or the PCR.	• Repeat the RNA isolation or real-time RT-PCR with fresh reagents and freshly decontaminated pipettes. • Set up the real-time RT-PCR in an area separate from areas used for RNA isolation and PCR product analysis.

Documentation and support

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)
  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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Revision history of Pub. No. MAN0008826 (English)

Revision	Date	Description
B.0	9 May 2017	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	10 April 2014	Baseline for revision history

Certificate of Analysis

Certificat d'Analyse

applied biosystems
by Thermo Fisher Scientific

VetMAX™ NDV Kit

Σ	100 Tests	LOT	NDV-065
REF	NDV		2025-12-12
			2026-11-18

COMPONENT DESCRIPTION *Description du composant*	REF	UNIT	LOT
VetMAX™ NDV Reagents	4406874	1 Box	2511067
2X RT-PCR Buffer	8732G	1375 µL	2510177
25X RT-PCR Enzyme Mix	2737G	110 µL	2510158
25X NDV Primer Probe Mix	1020G	110 µL	2511065
Nuclease Free Water	9914G8	1750 µL	2507148
TaqMan™ NDV and Xeno™ RNA Controls	4406875	1 Box	3359227
25X NDV Control RNA	1023G	15 µL	3358866
Xeno RNA Control (10000 copies/µL)	5716G	110 µL	3355301
Nucleic Acid Dilution Solution	5717G	500 µL	3355361

INSTRUCTIONS FOR USE *Notices d'utilisation*

French MAN0008825 RevB.0
English MAN0008826 RevB.0

Quality Assurance

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