Avian GEN Dx - Newcastle Disease Virus (NDV) RT-qPCR Detection kit

Instructions For Use

AVDX-NDV-100 100 Reactions

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Location: Microzone,
Suite 3, Faraday House,
King William Street,
Stourbridge,
DY8 4HD

T: +44(0)1384 444585

E: info@microzone.co.uk

W: microzone.co.uk

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Introduction

Newcastle Disease Virus (NDV) is a highly contagious avian virus that causes significant disease and economic losses in poultry. As many NDV vaccines are based on low-virulence lentogenic strains, differentiation between vaccine strains and pathogenic field strains is important for effective surveillance.

The Avian GEN Dx – Newcastle Disease Virus RT-qPCR Detection Kit is designed for the sensitive and specific detection of pathogenic NDV field strains, including mesogenic and velogenic strains. The assay uses primers and probe targeting a virulence-associated region within the fusion (F) gene and is designed to support differentiation between pathogenic field strains and commonly used lentogenic vaccine strains.

The kit contains all components required for RT-qPCR, including a lyophilised mastermix with Hot Start Taq DNA Polymerase, Reverse Transcriptase and RNase Inhibitor, an NDV Positive Control and NDV Detection Mix. It is compatible with real-time PCR instruments capable of detecting FAM-labelled probes and is suitable for molecular surveillance and outbreak investigation.

It is intended for research use only by trained laboratory staff.

Kit Contents

Product	Product Code	Quantity
2X MegaMix NDV Mastermix (Lyophilised)	MM-NDV-1	1 vial
MasterMix Reconstitution Buffer	LYORB-1	1 x 1 mL
NDV Detection Mix (Lyophilised)	NDV-DM-0.5	1 x 500 µL
NDV Positive Control	NDV-PC-0.1	1 x 100 µL
Just Water (Molecular Grade Water)	5JWA-1	1 x 1 mL
25 µM ROX Reference Dye	ROX-0.05	1 x 50 µL

Storage and Stability

Store the Avian GEN Dx Newcastle Disease Virus (NDV) RT-qPCR Detection kit at -20°C until the expiry date displayed on the label.

Materials Required but Not Provided

- Disposable powder-free nitrile gloves
- Pipettes (adjustable) and sterile filtered pipette tips
- 0.5/1.5 mL nuclease free microcentrifuge tubes
- Benchtop centrifuge
- Vortex mixer
- Decontaminated bench
- Optically clear PCR tubes and caps or 96-well microplate suitable for use with chosen PCR instrument
- Plate sealer (if required)
- qPCR Thermal cycler compatible with FAM fluorophore

Reagent Preparation

2X MegaMix NDV Mastermix

1. Bring 2X MegaMix NDV Mastermix (Lyophilised) vial to room temperature.
2. Remove bung and add 1 mL of Reconstitution Buffer (LYORB-1) to the 2X MegaMix NDV Mastermix vial.
3. Replace bung and incubate at room temperature for 30 minutes.
4. Mix well via inversion.
5. Aliquot into required volumes and store at -20°C, avoid multiple free thaw cycles.

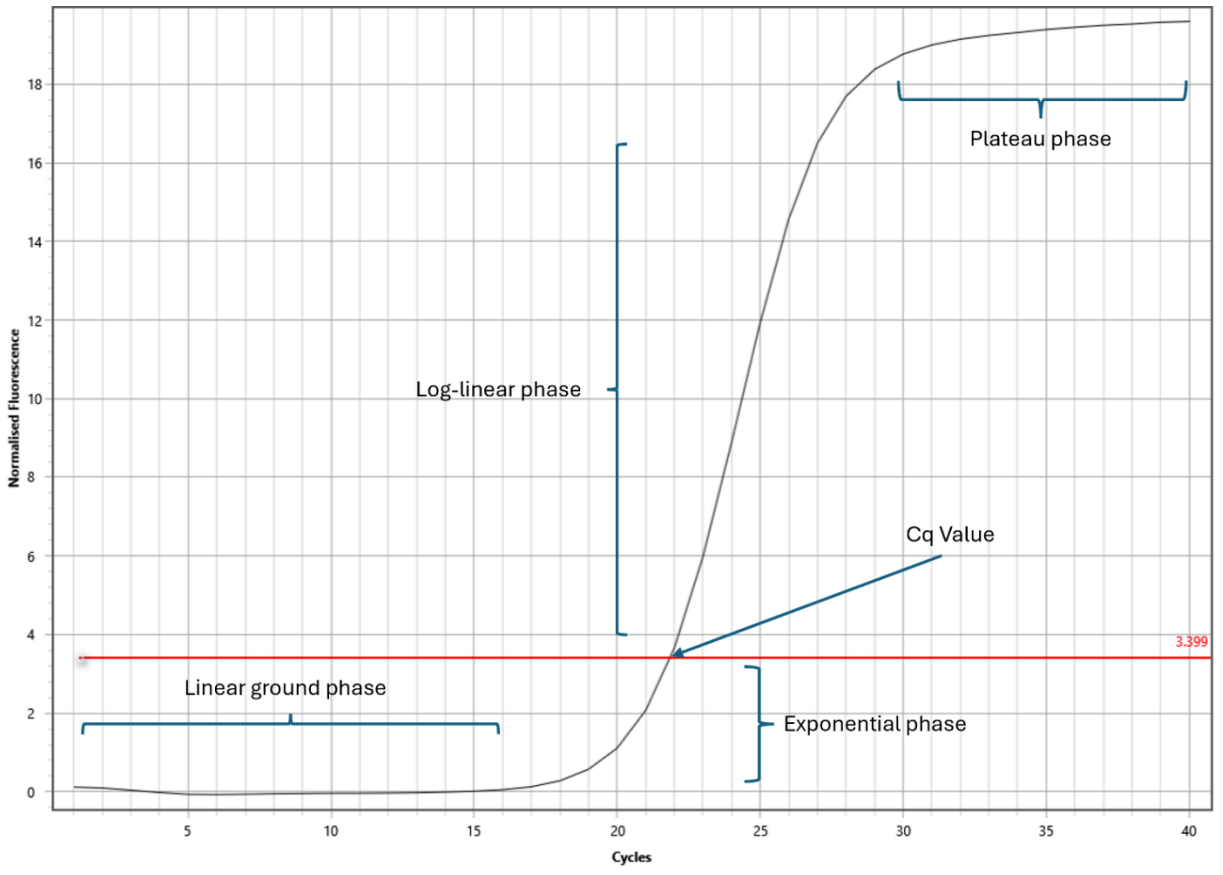
NDV Detection Mix

1. Bring NDV Detection Mix (Lyophilised) vial to room temperature.
2. Remove bung and add 0.5 mL of Just Water (Molecular Grade Water) to the NDV Detection Mix vial.
3. Replace bung and incubate at room temperature for 30 minutes.
4. Mix well via inversion.
5. Aliquot into required volumes and store at -20°C, avoid multiple free thaw cycles.

Assay Principle

The assay utilises RT-qPCR to detect the presence of specific RNA sequences in the target of interest. In the first step, the reverse transcriptase enzyme synthesises complementary DNA (cDNA) from the viral RNA template using specific primers. The cDNA is then converted into double-stranded DNA by DNA polymerase, which also drives the subsequent amplification process. During repeated PCR cycles, the target DNA is exponentially amplified, while fluorescent dyes or sequence-specific probes generate a signal proportional to the amount of DNA produced. This allows the detection and quantification of the original RNA in real time, providing both qualitative and quantitative information about the target sequence.

In summary, a qPCR amplification curve typically consists of four phases. First is the linear ground (baseline) phase, where the amount of amplified DNA is too low to produce a detectable fluorescence signal. This is followed by the exponential phase, where the target DNA doubles at each cycle and fluorescence rises proportionally. Next comes the linear (log-linear) phase, in which amplification efficiency begins to decline as reaction components become limiting. Finally, the plateau phase is reached, where amplification slows and fluorescence levels off as reagents are depleted.



Linear plot of a qPCR curve.

Technology

Dual-labelled hydrolysis probes are the most widely employed method in quantitative PCR (qPCR) for detecting and quantifying specific DNA sequences. It utilises a fluorescently labelled probe that binds to a complementary target sequence within the PCR product. The probe is a short oligonucleotide, consisting of a reporter dye at the 5’ end and a quencher at the 3’ end. While the probe is intact, the quencher suppresses the fluorescence of the reporter. During the PCR amplification, the 5' to 3' exonuclease activity of Taq polymerase cleaves the probe’s nucleotides, separating the reporter from the quencher, leading to an increase in fluorescence proportional to the amount of product amplified. The increase of fluorescence signal due to the dual-labelled hydrolysis probe technology allows the real-time monitoring of PCR amplification, providing highly specific and sensitive detection of nucleic acids.

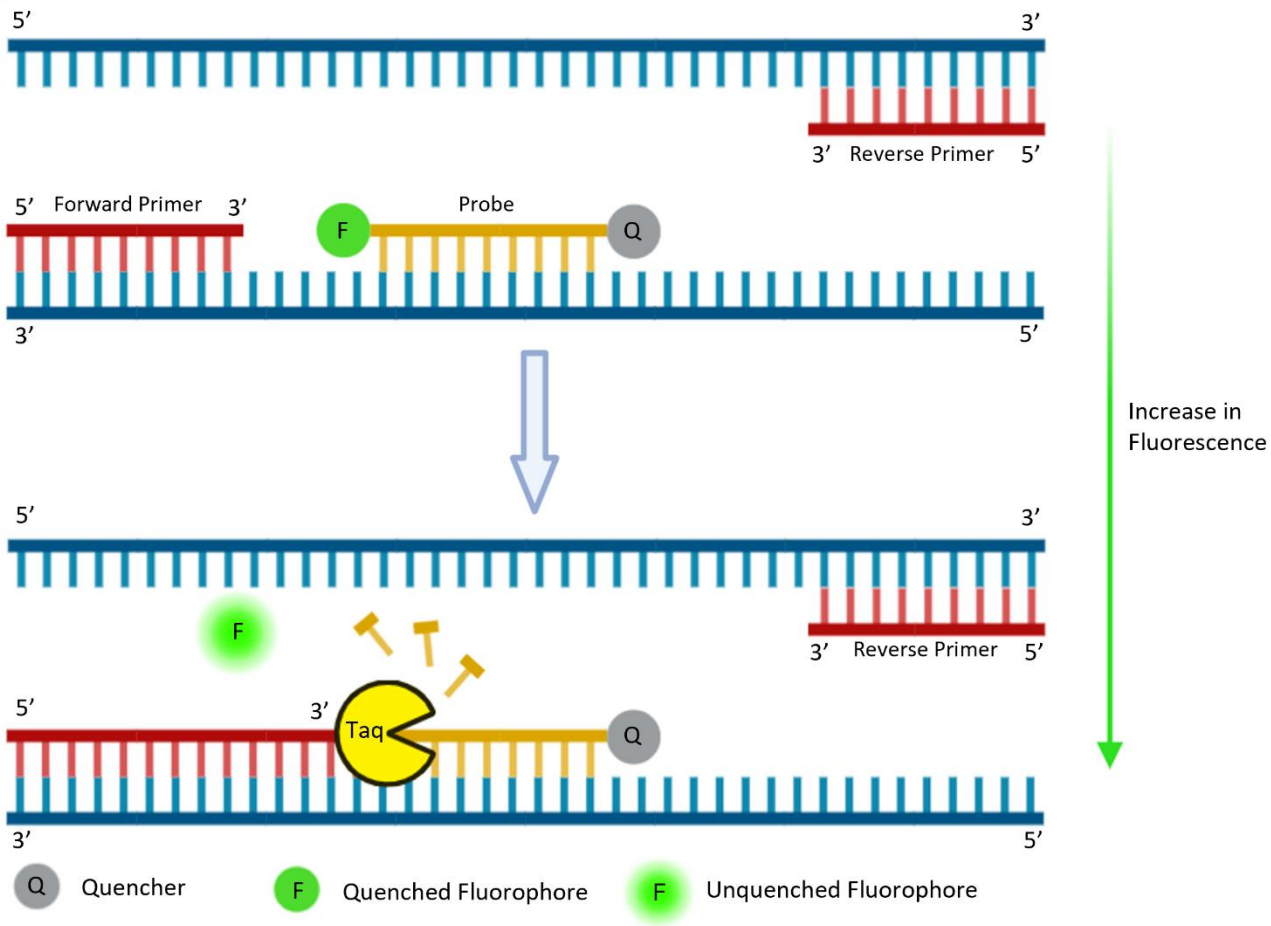


Diagram showing how dual-labelled hydrolysis probes work.

Assay Design

Avian GEN Dx - Newcastle Disease Virus (NDV) RT-qPCR Detection kit is designed to target the fusion protein (F) gene of the Newcastle Disease virus’s genome and can detect the presence of virus even in low copy numbers.

Protocol

The protocol consists of three steps: nucleic acid extraction/purification, RT-qPCR and analysis. Nucleic acids are extracted/purified from samples suspected of being Newcastle Disease Virus positive. The purified nucleic acids are then reverse transcribed and amplified using 2X MegaMix NDV Mastermix and NDV Detection Mix in the same reaction. Analysis of the RT-qPCR amplification allows the user to determine if the tested sample contains Newcastle Disease Virus's nucleic acids.

Nucleic Acid Extraction/Purification

This kit can be used with traditional nucleic acid extraction methods that utilise magnetic beads or columns and alternatively, it can be used with a direct-to-PCR lysis buffer such as microLYSIS PLUS RNA from Microzone. microLYSIS PLUS RNA significantly shortens the sample preparation process, with only a short heat step to release, concentrate and stabilise nucleic acids ready for RT-qPCR. More information about microLYSIS RNA contact Microzone.

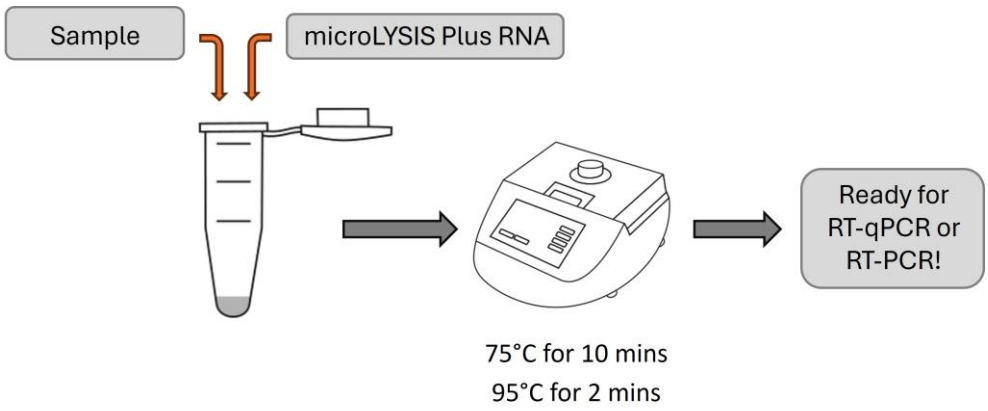


Diagram showing sample preparation with microLYSIS PLUS RNA workflow.

Reaction Setup

1. Reconstitute the 2X MegaMix NDV Mastermix (Lyophilised) and NDV Detection Mix (Lyophilised) as described in Reagent Preparation section.
2. Thaw all reagents at room temperature.
3. Once thawed vortex all reagents and briefly centrifuge to remove any liquid from the cap.
4. Every reaction set up must allocate sufficient tubes for the number of samples, a positive, and a negative control (samples + 2 = total tubes needed). For example, 10 samples to test + 1 Positive control tube + 1 negative control tube = 12 reaction tubes required.

5. Setup should be done on ice when possible. New sterile filter pipette tips are to be used between each pipetting.
6. Generate the following mastermix, use the number of reactions tubes determined in step 4, adding 5% excess (to allow for pipetting loss) to calculate volumes:

Component	Volume
2X MegaMix NDV Mastermix	10 µL
NDV Detection Mix	5 µL
Total volume of mastermix per sample	**15 µL**

Optional ROX Addition to mastermix: For low-ROX instruments, add ROX to a final concentration of 50 nM (0.04 µL of 25 µM ROX per reaction). For high-ROX instruments, use a final concentration of 500 nM (0.4 µL per reaction). Refer to the instrument manufacturer's instructions to confirm the required ROX concentration.

7. Briefly vortex to mix.
8. Dispense 15 µL of mastermix into each PCR tube.
9. Add 5 µL of sample to PCR tube containing mastermix; for the positive control and negative control add 5 µL NDV Positive Control and 5 µL Just Water (Molecular Grade Water), respectively.
10. Cap the tube with corresponding cap.
11. Briefly centrifuge to move all of the reaction to the base of the tube (some instruments this is not required as it is done during the PCR).
12. Transfer the tubes to the PCR instrument.

Thermal Cycling

Run the following thermal cycling profile:

Temperature	Time	Cycles	Fluorescence collection	Step
50°C	15 minutes	1		Reverse Transcription
95°C	2 minutes	1		Initial Denaturation
95°C	15 seconds	40		Denaturation
60°C	30 seconds		FAM	Annealing/Extension

Interpretation of Results

Target	Reporter Fluorophore	Sample	
		Cq Value	Interpretation
Newcastle Disease Virus (NDV)	FAM	<38	Positive
		≥38 or no amplification	Negative

	Newcastle Disease Virus (FAM)	Reporting
Positive Control	+	Valid
Negative Control	-	Valid
Test 1	+	Positive for NDV
Test 2	-	Negative (NDV Free)
Positive Control	-	Invalid Run / Retest
Negative Control	+	Invalid Run / Retest