

MBPCR126

Hi-PCR[®] Newcastle Disease Virus (NDV) SYBr PCR Kit

Description

Newcastle disease virus (NDV) – A type strain for avian paramyxoviruses. Members of this family have a single-stranded, linear, RNA, with an elliptical symmetry. The total genome is roughly 16,000 nucleotides. NDV is a contagious and fatal viral disease affecting most species of birds. Clinical signs are extremely variable depending on the strain of virus, species and age of the bird, concurrent disease, and preexisting immunity. NDV is so virulent that many birds die without showing any clinical signs. A death rate of almost 100 percent can occur in unvaccinated poultry flocks. NDV can also infect and cause death even in vaccinated poultry. NDV is spread primarily through direct contact between healthy birds and the bodily discharges of infected birds. Hence the diagnosis of such a virulent strain is of utmost importance to save the life of many birds.

NOTE: HiMedia's Hi-PCR[®] Newcastle Disease Virus (NDV) SYBr PCR Kit is for *in-vitro* use only.

Intended Use:

Recommended for sensitive and specific detection of Newcastle Disease Virus in animal samples.

Principle

HiMedia's Hi-PCR[®] Newcastle Disease Virus (NDV) SYBr PCR Kit is designed for detection of the matrix (M) gene of NDV using specific primers. This kit allows rapid, sensitive, and specific detection of NDV. This kit also contains **Positive control**.

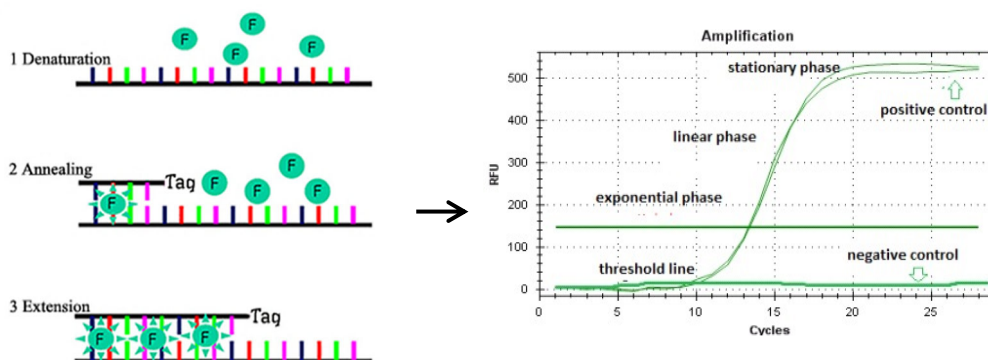
Positive control

This is a control reaction using a known template (target pathogen). A positive control is usually used to check that the primers have been designed properly and the PCR conditions have been set up correctly.

Principle

Real-time PCR also called quantitative PCR (qPCR) or kinetic PCR, is a laboratory technique based on the principle of PCR. This technique is used to amplify and simultaneously quantitate a targeted DNA sequence. The presence of SYBr Green Dye, a dsDNA-binding dye in the Hi-SYBr Master Mix allows for simplified assay design without the need for additional fluorescent probes and enables assay verification using a melt-curve analysis. HiMedia's Hi-PCR[®] Newcastle Disease Virus (NDV) SYBr PCR Kit is a qualitative Real-Time PCR kit which includes the amplification of the matrix (M) gene.

Diagrammatic representation of SYBr Green Chemistry in Real-Time PCR



The SYBr Green dye cycles between an unbound (Denaturation step) and a bound (Annealing through Extension) state as the reaction progresses. Signal intensity increases as the quantity of amplicons increase in later cycles indicating amplification. During elongation, more and more dye molecules bind to the newly synthesized DNA. If the reaction is monitored continuously, an increase in fluorescence is viewed in real-time. Upon denaturation of the DNA for the next heating cycle, the dye molecules are released and the fluorescence signal falls.

Features

- Fast and simple
- Sensitive and specific results
- Guaranteed reproducible results
- Rapid detection of all relevant clinical pathogens

Sample Source: Serum, tissue samples

Storage and Shelf-life

The provided kit has a shelf-life of 12 months when stored between -10° to -20°C . Repeated thawing and freezing of PCR reagents should be avoided as this may reduce the sensitivity. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. Degradation of sample DNA specimens can also reduce the sensitivity of the assay. HiMedia does not recommend using the kit after the expiry date stated on the pack.

Kit Contents

The provided PCR Kit contains:

Components	Product code	Reagents provided for (reactions)*(μL)	
		25R	50R
Hi-SYBr master mix	MBT074	270	540
One Step RT Enzyme Mix	DS0286	27	54
Primer Mix for NDV	DS0244A	27	54
NDV Positive Control	DS0273	25	50
Molecular Biology Grade water for PCR	ML065	100	200

*For a 20 μL PCR reaction

Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19) & Sealing film (PR18)
- Insta Q Real Time PCR System (Product Code: LA1012 / LA1023 / LA1024 / LA1073 / LA1074)
- Barrier Micropipette Tips (Product Code: LA749 / LA749A / LA751 / LA751A / LA750 / LA750A / LA859 / LA859A)
- Micropipettes
- Materials needed but not provided:

- PCR tubes (Product code PW1255) or PCR Strips (Product code: PR17) or PCR Plates (Product code: PR2 / PR3 / PR19) & Sealing film (PR18)
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- Micropipettes
- HiPurA® Multi-Sample DNA Purification Kit (MB615)

Specimen collection and Handling

Follow appropriate techniques for handling specimens; after use, contaminated materials must be sterilized by autoclaving before discarding. Standard precautions as per established guidelines should be followed while handling clinical specimens and items contaminated with blood and other body fluids. Safety guidelines may be referred to individual safety data sheets.

General Preparation Instructions

- Before use, a suitable amount of all PCR components should be completely thawed on ice (4°C).
- Perform the amplification reactions in a clean area.
- Use of aerosol barrier pipette tips is recommended to reduce contamination risks from extraneous RNA/DNA templates.
- Extract and store positive control material (if used) separately from all other reagents to avoid contamination and add it to the reaction mix in a separate area.
- Centrifuge the components briefly once thawed.

A. Protocol for PCR Master Mix Preparation

Perform PCR reactions for each RNA sample as per the following table:

Components	Recommended volume to be added per reaction (µL)
Hi-SYBr master mix (MBT074)	10
One Step RT Enzyme Mix	1
NDV Primer Mix	1
Template RNA (Extracted RNA)/ NDV Positive Control/Negative Control	5
Molecular Biology Grade water for PCR	Up to 20

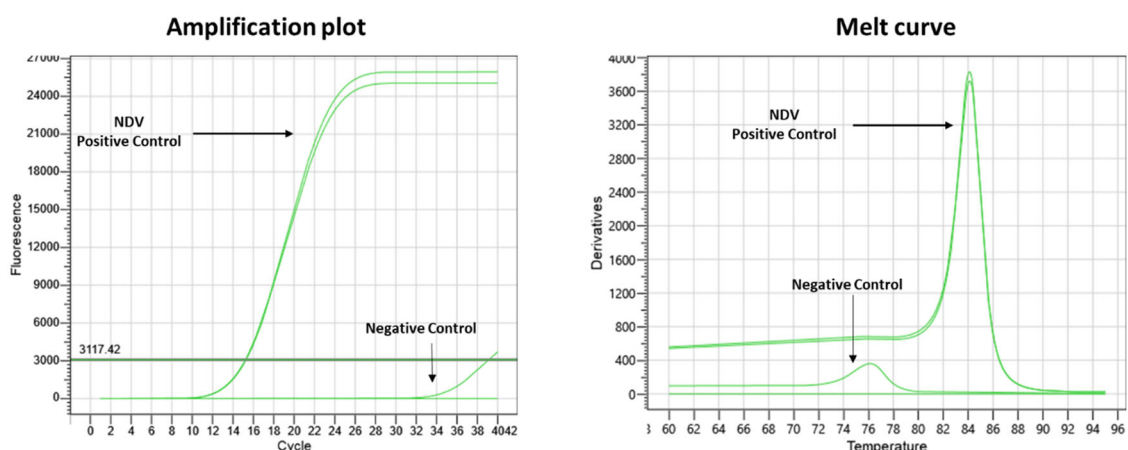
Centrifuge the tube briefly at 6000 rpm for about 10 seconds and place the tubes in the PCR machine and set the recommended PCR program (mentioned below). Interpret the data from the amplification plot (observe the Ct values).

B. Recommended PCR program

1. Reverse Transcription : 50 °C for 15 minutes No. of cycles: 1
2. Initial denaturation : 95°C for 2 minutes 30 seconds No. of cycles: 1
3. Denaturation : 94°C for 10 seconds
4. Annealing (Plate Read) : 55°C for 45 seconds } No. of cycles: 40
5. Melt Curve Analysis as per HiMedia's Insta Q96 Real Time PCR Machine
 - a. 95°C : 15 seconds
 - b. 60°C : 1 minute
 - c. 95°C : 15 seconds
 - d. Increment : 0.5°C
 - e. On Hold : 10 seconds

NOTE: The user can also set up a melt curve program as per their existing PCR instrument.

C. Amplification data



Sr. No.	Sample	C _t value	T _m Value
1	Positive control	15.4	84.1
2	Negative control	N/A	76.2 or No T _m

Data interpretation

Melting Temperature (T _m)*	Result Interpretation
82°C - 86°C	Positive for NDV

Warning and Precautions

Not for Medicinal Use. Read the procedure carefully before starting the experiment. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred to in the safety data sheets of the product.

Performance and Evaluation

Each lot of HiMedia's Hi-PCR® Newcastle Disease Virus (NDV) SYBr PCR Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Each lot of HiMedia's Hi-PCR® Newcastle Disease Virus (NDV) SYBr PCR Kit is assayed for contaminating endonuclease, exonuclease and non-specific RNase / DNase activities. Functionally tested for amplification.

Troubleshooting Guide

Sr. No.	Problem	Cause	Solution
1.	No amplification	Degraded samples	1. Check the integrity of RNA using agarose gel electrophoresis. 2. Use freshly prepared RNA to ensure the availability of intact template sequence for efficient amplification.
		Error in protocol setup	Verify that the correct reagent volumes, dilutions and storage conditions have been used.
2.	Variability between replicates	Error in reaction set-up	Prepare large volume reaction mix, vortex thoroughly and aliquot appropriately into reaction tubes.
		Air bubbles in reaction mix	Briefly centrifuge reaction samples/plate prior to running on a PCR machine.

		Pipetting error	Replicates can show increased variation due to poor laboratory techniques or imprecise pipettes.
3.	Amplification in negative control	Reagents contaminated	<ol style="list-style-type: none"> 1. Replace all critical solutions. 2. Repeat the analysis of all tests with fresh aliquots of critical reagents.

Safety Information

HiMedia's Hi-PCR® Newcastle Disease Virus (NDV) SYBr PCR Kit is for laboratory use only, not for drug, household, or other uses. Take appropriate laboratory safety measures and wear gloves when handling.









Disposal

The user must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com.

Symbols

	Manufacturer		Do not use if package is damaged
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

Identification No.: PIMBPCR126

Rev.No.:06

Date of Issue: 2025-04

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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