INSTRUCTIONS FOR USE

ID GENE™ NEWCASTLE DISEASE DUPLEX

REAL-TIME RT-PCR ASSAY FOR THE QUALITATIVE DETECTION OF NEWCASTLE DISEASE VIRUS RNA

PATHOGEN	Newcastle Disease Virus
NUCLEIC ACID TYPE	RNA
SPECIES	Birds
SAMPLE TYPES	• **Swabs** (tracheal, oropharyngeal and cloacal swabs; Individual samples or pools of up to 5) • **Organ** (trachea, lung, spleen, liver, brain and cecal tonsils; Individual samples or pools of up to 5)) • **Nucleic acid storage card** (individual samples or pools of up to 5)
ASSOCIATED PRODUCTS	• ID Gene™ Mag Fast Extraction Kit (MAGFAST384) • ID Gene™ Spin Universal Extraction Kit (SPIN50 / SPIN250) • NDV Positive Extraction Control (PEC-NDV)
PRODUCT CODES AND FORMATS	**IDNDV-50** 50 tests **IDNDV-100** 100 tests

In vitro use

IDNDV version 1019 EN

GENERAL INFORMATION

Characteristics

ID Gene™ Newcastle Disease Duplex (IDNDV) kit is a RT-qPCR kit that amplifies a target sequence in Newcastle Disease Virus (NDV) genome.

This kit is a qualitative duplex test. It simultaneously amplifies target RNA and an exogenous non-target positive control.

This kit can be used to test swab (tracheal, oropharyngeal and cloacal), organ (trachea, lung, spleen, liver, brain and cecal tonsil) from birds or nucleic acid storage card. Either individual samples or pools of up to 5 may be tested.

Kit composition and storage conditions

The IDNDV kit contains the reagents shown below:

REFERENCE	COMPONENT	VOLUME	DESCRIPTION
PAC-NDV	Positive Amplification Control	100 µl 1 tube (blue cap)	NDV-specific synthetic nucleic acids
NTPC-BIRD	Exogenous Non-Target Positive Control	2200 µl 1 vial	Non-pathogenous microorganism, mime of the target. Freeze-dried pellet to be reconstituted in **2200 µl** distilled or Nuclease-free water.
ARM-NDV	Amplification Reaction Mix	400 µl 1 / 2 tubes (white cap)	Ready-to-use reaction mixture containing the Reverse Transcriptase, the Taq polymerase, primers, hydrolysis probes and oligonucleotides for the amplification and detection of NDV genome and the exogenous non-target positive control.

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) in order to avoid more than 3 freeze/thaw cycles.

Materials required but not provided in the kit

All material used should be of suitable quality for molecular biology.

Instruments:

- Real-time PCR thermal cycler with channels capable of reading the following fluorophores: FAM™ and HEX™or VIC®.

Equipment, consumables and reagents:

- Precision pipettes capable of delivering volumes of between 1 µl and 1000 µl.
- Nuclease-free filtered tips.
- 1.5 ml tubes.
- 96-well PCR plates or PCR tubes (that have an optical quality compatible with the thermal cycler) and suitable adhesive films or caps.
- Refrigerated rack.
- Distilled or Nuclease (RNAse and RNAse)-free water

EXTRACTION AND AMPLIFICATION CONTROLS

The following controls are suggested to be used in each run:

NDV Positive Extraction Control (PEC-NDV), available separately

This is a freeze-dried control which consist of an inactivated Newcastle Disease Virus strain diluted into tracheal and oropharyngeal swabs supernatants from negative birds.

This control is prepared and extracted in the same way as samples, to validate the efficiency of the nucleic acid extraction and the RT-qPCR amplification processes. The PEC-NDV may be used to monitor variations in analytical sensitivity. Please refer to the dedicated Quality Control Data Sheet & instructions for use.

NDV Positive Amplification Control (PAC-NDV)

This control validates the amplification of the target. It consists of a synthetic nucleic acid target.

Note: If the PEC-NDV, or an internal sentinel sample, is already used as a positive control for the test runs, the use of the PAC-NDV is not mandatory.

Exogenous Non-Target Positive Control (NTPC-BIRD)

The exogenous positive control is a non-pathogenous microorganism, acting as a mimic of the target.

To evaluate the efficiency of the extraction process and to detect the presence of inhibitors in the amplification step.

This control is to be added to every sample as well as to the other controls (PEC-NDV and NEC) before extraction.

Negative Extraction Control (NEC)

This control is expected not to contain the target pathogen. It must be processsed in the same way as samples, as for pre-treatment, when necessary, and nucleic acid extraction.

The sample is here replaced by either a **negative matrix (NEC-matrix) or Nuclease-free water (NEC-H_2O).**

*Note: When using a **NEC-matrix**, refer to the extraction kit protocol for the matrix in question.*

Negative Amplification Control (NAC)

This control contains 8 µl of reaction mix (ARM-NDV) and 5 µl of Nuclease-free water. It is included in each run to control for the absence of aerosol contaminants.

NUCLEIC ACID EXTRACTION

The RNA of Newcastle Disease Virus must be extracted from the sample before being amplified by real time RT-PCR.
For optimal results, we recommend using the following IDvet extraction kits:

DESCRIPTION	PRODUCT NAME	PRODUCT CODE
Magnetic bead extraction system	ID Gene™ Mag Fast Extraction Kit	MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50 / SPIN250

Important note

While IDvet's qPCR tests may be used with extraction kits from other suppliers, it is important to contact info@id-vet.com BEFORE running your tests in order to verify compatibility.

AMPLIFICATION PROTOCOL

Preparation of the RT-qPCR amplification reaction

1. Prepare an experimental plan for the analysis of the samples and controls, being sure to distance the positive control (PAC-NDV) from the other samples.
2. Thaw the IDNDV kit, ideally at 5°C (± 3°C) in a refrigerated rack. Thaw at room temperature (21°C ± 5°C) only if the mix is to be used immediately after thawing.
3. Homogenize the contents of the **ARM-NDV** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-NDV** per well. Use PCR plates or PCR tubes adapted to the thermal cycler in use.
5. Add the following to the reaction mix:
   - 5 µl of RNA extracted from each sample to be analyzed
   - 5 µl of PAC-NDV
   - 5 µl of extracted NEC
   - 5 µl of nuclease-free water (NAC)
6. Cover the plates or tubes with suitable adhesive films or caps.

Programming the amplification phase

1. Program the thermal cycler detectors to read the following wavelengths for each well:

TARGET	CHANNEL CAPABLE OF READING	QUENCHER*
NDV	FAM™	non fluorescent
Exogenous Non-Target Positive Control (NTPC-BIRD)	VIC®/HEX™	non fluorescent (compatible VIC®/HEX™)

Note: For devices requiring an internal reference for optical calibration, the amplification mix contains ROX.
** For the quencher, setting instrument parameters for TAMRA™ dye instead of non fluorescent dye may improve the data analysis with some instruments.*

2. Choose between the two different amplification programs validated by IDvet:
   - Standard program (which allows PCR kits from different vendors to be used in a single run)
   - or Rapid program

STEP	STANDARD PROGRAM	RAPID PROGRAM	NUMBER OF CYCLES
(1) Reverse transcription	10 min at 45°C	10 min at 45°C	1
(2) Polymerase activation	10 min at 95°C	2 min at 95°C	1
(3) DNA denaturation/elongation	15 sec at 95°C 60 sec at 60°C	10 sec at 95°C 30 sec at 60°C	40

Note: The fluorescence is read at the end of the elongation phase at 60°C.

3. Enter one of these programs in the thermal cycler and select a final volume of **13 µl per PCR**. If different volumes are combined in a single run, enter the largest volume used on the same run.
4. Place the PCR tubes or PCR plates in the thermal cycler and start the program

VALIDATION AND INTERPRETATION OF RESULTS

Assay validation

The analysis of results is based on the Cq (Quantification cycle) value of each sample that is obtained by each detector. The Cq is also known as the Ct value (Threshold cycle).

The test is validated according to criteria outlined in the table below. **Results should not be interpreted if any of these criteria are not met.**

CONTROL	ADDITION OF NTPC-BIRD	EXPECTED RESULT	ACCEPTABILITY CRITERIA
PAC-NDV	-	Detected in FAM™	• Presence of a characteristic curve • Refer to the Cq value given in the quality control certificate
NEC	+	Detected in VIC®/HEX™	• Presence of a characteristic curve in **VIC®/HEX™** • Refer to the Cq value given in the quality control certificate
NAC	-	No detection	Absence of a characteristic curve

Suggested interpretation of results

For each sample, results may be interpreted according to the following criteria:

NDV SIGNAL	NTPC-BIRD SIGNAL	INTERPRETATION
Detected	Detected or not detected	Animal detected as positive for **NDV**
Not detected	Detected	Animal not detected for **NDV**
Not detected	Not detected or when $Cq_{sample} > Cq_{NEC\text{-}matrix} + 3$ for NTPC-BIRD signal	A problem occurred during sample distribution or extraction processes and/or PCR reaction was inhibited

Non-validated samples:

- **If the NTPC-BIRD is not detected but the sample is detected positive for NDV, consider the sample as positive**.
- **If $Cq_{NTPC\text{-}BIRD} > Cq_{NEC} + 3$ and no signal is detected for NDV, the reaction was inhibited**.
  In this case, prepare a new amplification run following the procedure below:
  1. Dilute the extracted RNA 10 times in nuclease-free water.
  2. Repeat the amplification using 5 µl of this dilution.
  3. If the NTPC-BIRD is detected, interpret the sample according to table above.
  4. If the NTPC-BIRD is not detected, re-extract the sample or consider it uninterpretable.

Risks and precautions of use

Some components contain hazardous substances. Wear protective gloves / protective clothing / eye protection.
Material and Safety Data Sheets (MSDS) are available upon request.

Technical support and Documentation

For all questions, technical support, requests for MSDS and protocols, please contact us at the following address: info@id-vet.com

History of revisions

VERSION	EDIT DATE	REFERENCE	TYPE OF VERSION	REVISION MADE
1019	12/2019	DOC2063	Not applicable (first version)	N/A