Certified management system

ID Gene™ Neospora caninum Duplex

Ref: IDNEO-50 / IDNEO-100

50 / 100 tests

Real-time PCR for the qualitative detection of *Neospora caninum*

Suitable samples: brain, placenta, muscle

***In-vitro* Use**

New protocol version

- **December 2016: Changing the PCR sample volume**
- **Novembre 2016: substitution of plasmid (PLS-NEO) with a lyophilized positive control (TPC-NEO)**

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General information

▪ Characteristics

ID Gene™ Neospora caninum Duplex (IDNEO) is a real-time PCR kit that amplifies a target sequence in *Neospora caninum* genome, causative agent of Neosporosis.

This kit is a **qualitative duplex test**. It simultaneously amplifies target DNA and endogenous internal control.

The kit contains the target positive control (TPC-NEO) which are to be extracted in the same manner as the samples to evaluate extraction and amplification steps.

This kit can be used to test brain, placenta or muscle homogenates.

▪ Kit composition and storage conditions

The IDNEO kit contains the reagents shown below:

Reference	*Component*	*Volume*	*Description*
TPC-NEO	Target Positive control	550 µl 1 vial	NEO-positive mash of brain sample, freeze-dried and calibrated between 10 and 100 times the detection limit of the method (MDL). Freeze-dried pellet to be reconstituted in **550 µl** distilled or Nuclease-free water.
ARM-NEO	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mixture containing Taq polymerase and oligonucleotides for detection of *Neospora caninum* and of the endogenous non-target positive control.

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) such as not to thaw components more than 3 times.

▪ Required equipment, not provided in the kit

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

Amplification Instrument:

Real-time thermal cycler capable of reading the following wavelengths: 525 nm (FAM), 548 nm (Yakima Yellow, equivalent to VIC).

Examples of compatible thermal cyclers: CFX96, Chromo4 Biorad, LC480 I, LC480 II, LC96 Roche, 7500 AB, and Rotorgen Qiagen. Please contact us for use with other thermal cyclers.

Consumables:

- Precision pipettes capable of delivering volumes of between 1 µl and 1000 µl with Nuclease-free filtered tips
- 1,5 ml tubes
- 96-well PCR plates, strips or PCR micro-tubes (that have an optical quality compatible with the thermal cycler) and appropriate adapted adhesive film or caps

Reagents:

- Distilled or Nuclease-free water;

Remarks and precautions

The material used contains less than 0,1% hazardous or carcinogenic substances, thus MSDS sheets are not required. However, it is recommended to take appropriate precautions, as with any biochemical product, and to wear appropriate clothing.

Extraction and amplification controls

▪ Positive controls

The IDNEO kit contains the following positive controls:

- Target Positive Control (TPC-NEO):

This control consists of positive mash of brain sample, calibrated between 10 and 100 times the detection limit of the method (MDL).
This control validates the extraction and amplification of the target.
This control is prepared and extracted in the same way as samples.

- Endogenous Non-Target Positive Control (NTPCen):

This control is constitutively present in the cells of the test sample. It validates cell lysis and amplification of a non-target gene. It also confirms the presence of cells, and gives an indication of the state of the sample.

▪ Negative controls

It is recommended to include the following negative controls:

- Negative extraction control (NEC)

It is recommended to include a negative control for extraction (**NEC)** in each run.
This control is to be prepared and extracted in the same way as samples, but does not contain any target DNA. The volume occupied by the sample is replaced by a non-reactive matrix or Nuclease-free water.

- Negative control for amplification (NAC)

This control contains 8 µl of reaction mix (ARM-NEO) and 5 µl of Nuclease-free water. It is included in each analysis cycle to control for the presence of any aerosol contaminants.

Amplification protocol

▪ Extraction of DNA

The DNA of *Neospora caninum* must be extracted from the sample before being amplified by PCR.
For this, IDvet Genetics offers a range of extraction kits that conform to the French NF U47-600 standard:

Description	*Product name*	*Product code*
Magnetic bead extraction system	ID Gene™ Mag Universal Extraction Kit ID Gene™ Mag Fast Extraction Kit	MAG192/MAG384 MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50/SPIN250

▪ Preparation of the real-time PCR amplification reaction

1. Prepare an experimental plan for the analysis of the samples and controls, being sure to distance the positive control (TPC-NEO) from the other samples.
2. Thaw the IDNEO 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. Homogenise the contents of the **ARM-NEO** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-NEO** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Add :
   - 5 µl of DNA extracted from each sample to be analyzed
   - 5 µl of TPC-NEO
   - 5 µl of extracted NEC
   - 5 µl of Nuclease-free water (NAC)
6. Cover the plate or strips with appropriate adhesive film or caps.

▪ Programming the amplification phase

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

Target	*Fluorophore*	*λ (nm)*	*Quencher*
NEO	FAM	495-525	non fluorescent
NTPCen	VIC / Yakima Yellow	426-548	non fluorescent (compatible VIC/HEX)

Note: For devices requiring an internal reference, the amplification mix IDNEO contains ROX.

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

Step	*Standard program*	*Rapid program*	*Number of cycles*
(1) Polymerase activation	10 min at 95°C	2 min at 95°C	1
(2) 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 reading is taken at the end of the elongation phase at 60°C.

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

Validation and interpretation of results

- **Assay validation**

The analysis of results is based on the Cq value of each sample that is obtained by each detector.
The test is validated according to criteria outlined in the table below. **The result cannot be reliably interpreted if any of the criteria are not met**.

Control	*Expected result*	*Acceptability criteria*
TPC-NEO	Detected in FAM	Presence of a characteristic curve
NTPCen	Detected in VIC in each sample	Presence of a characteristic curve
NEC	Nothing detected if water used	Complete absence of a characteristic curve
	Detected in VIC if virus-negative sample used	Presence of a characteristic curve
NAC	No detection	Complete absence of a characteristic curve

- **Suggested interpretation of results**

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

NEO signal	*NTPCen signal*	*Interpretation*
Detected	Detected or Not detected	Animal detected as positive for Neosporosis
Not detected	Detected	Animal not detected for Neosporosis
Not detected	Not detected	A problem occurred during sample distribution or extraction process / PCR reaction was inhibited

Non-validated samples:

- If the NTPCen is not detected but the sample is detected positive for NEO, consider the sample as positive.

- If the NTPCen is not detected:

- A problem occurred during sample distribution or during the extraction process. In this case, the sample is to be extracted again.
- Or the PCR reaction was inhibited. In this case, perform a new amplification run following the procedure below.

Procedure to follow if the PCR reaction was inhibited:

1. Dilute the extracted DNA 10-fold in Nuclease-free water.
2. Repeat the amplification phase on 5 µl of this dilution.
3. If the NTPCen is detected, interpret the sample according to table above.
4. If the NTPCen is not detected, re-extract the sample or consider it uninterpretable.

Documentation and support

For questions or technical support, please contact: **genetics@id-vet.com**

For additional information, visit **www.id-vet.com**