

ID Gene™ *Anaplasma phagocytophilum* Duplex

Ref: IDANA-50 / IDANA-100

50 / 100 tests



Real-time PCR assay for the **qualitative** detection of ***Anaplasma phagocytophilum***

Suitable samples: Ruminant whole blood, swabs (cervical, vaginal or placenta) or organ (spleen)

In-vitro Use



December 2017
Kit with a lyophilized Target Positive Control (TPC-ANA) (previously PLS-ANA)

General information

▪ Characteristics

ID Gene™ Anaplasma phagocytophilum Duplex (IDANA) is a real-time PCR kit that amplifies a target sequence in *Anaplasma phagocytophilum* genome, causative agent of Anaplasmosis.

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

The kit contains a target positive control (TPC-ANA) which is to be extracted in the same manner as the samples to validate the extraction and amplification of the target.

This kit can be used to test whole blood collected in EDTA , swabs (cervical, vaginal or placenta) or organ (spleen) of ruminants.

▪ Kit composition and storage conditions

The IDANA kit contains the reagents shown below:

<i>Reference</i>	<i>Component</i>	<i>Volume</i>	<i>Description</i>
TPC-ANA	Target Positive control	550 µl 1 vial	Bovine whole blood sample positive for Anaplasmosis diluted in a freeze-dried negative bovine blood 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-ANA	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mix containing Taq polymerase, oligonucleotides for amplification and detection of <i>Anaplasma phagocytophilum</i> and the endogenous 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 multiple freeze/thaw cycles (> 3 not recommended).

▪ Materials required but not provided in the kit

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

Amplification Instrument:

- Real-time thermal cycler with channels capable of reading the following fluorophores: FAM, HEX or VIC and Cy5.

Examples of compatible thermal cyclers: CFX96™, Chromo4™ (Biorad), LC®480 I, LC®480 II, LC®96 Roche, AB® 7500 and Rotor-Gene Q Qiagen. Please contact us regarding suitability with other thermal cyclers

Consumables:

- 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, strips or PCR micro-tubes (that have an optical quality compatible with the thermal cycler) and appropriate adapted adhesive film or caps
- Refrigerated rack

Reagents:

- Distilled or Nuclease-free water (recommended)

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 controls

▪ Positive controls

The IDANA kit contains the following positive controls:

- Target Positive control (TPC-ANA):

This control consists of bovine blood positive sample (*Anaplasma phagocytophilum*) diluted in bovine negative blood, calibrated between 10 and 100 times the MDL.

This control validates the efficiency of the extraction and amplification of the process.

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 sample to be tested. Its function is to validate (1) cell lysis and (2) amplification of a non-target gene. It also confirms the presence of cells, and gives an indication of the quality of the sample.

▪ **Negative controls**

It is recommended to include the following negative controls in each run:

- **Negative extraction control (NEC)**

This control should be prepared and extracted in the same way as samples, but should 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-ANA) and 5 µl of Nuclease-free water. It is included in each analysis cycle to control for the presence of any aerosol contaminants.

▪ **Extraction of the DNA**

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

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

▪ **Extraction of control**

Volume of the control to extract is described in the table below:

Important:

- Volume indicated is valid regardless of the extraction system.
- Control must be extracted at the same time as the samples.

Control	Volume
TPC-ANA	50 µl

Note: If the NEC is prepared with a negative sample matrix, refer to the extraction kit protocol for the matrix in question.

Amplification protocol

▪ **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-ANA) from the other samples.
2. Thaw the IDANA 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-ANA** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-ANA** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Add the following to the reaction mix
 - 5 µl of DNA extracted from each sample to be analyzed
 - 5 µl of TPC-ANA
 - 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	Channel capable of reading	Quencher
<i>Anaplasma phagocytophilum</i>	FAM	non fluorescent*
NTPCen	HEX/VIC	non fluorescent* (compatible VIC/HEX)

Note: For devices requiring an internal normalization of the signal, the amplification mix contains ROX.

**Using a TAMRA™ quencher can improve the data analysis with some instruments.*

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	10 sec at 95°C	40
	60 sec at 60°C	30 sec at 60°C	

Note: The fluorescence is read at the end of the elongation step 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 run, 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 (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	Expected result	Acceptability criteria
TPC-ANA	Detected in FAM and HEX/VIC	Presence of a characteristic curve Refer to the Cq value indicated in the quality control certificate
NTPCen	Detected in HEX/VIC in each sample	Presence of a characteristic curve
NEC	Nothing detected if water is used Detected in HEX/VIC if bacterial-negative sample used	Absence of a characteristic curve Presence of a characteristic curve
NAC	No signal	Absence of a characteristic curve

Note: TPC-ANA may be used to monitor variations in analytical sensitivity as it is calibrated at between 10 and 100 times the MDL.

Suggested interpretation of results

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

Sample	ANA signal	NTPCen signal	Interpretation
Individual	Detected	Detected or Not detected	Animal detected as positive for <i>Anaplasma phagocytophilum</i>
	Not detected	Detected	Animal detected as negative for <i>Anaplasma phagocytophilum</i>
	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 as positive for *Anaplasma phagocytophilum*, 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 times 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: support.genetics@id-vet.com

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