IDvet GENETICS

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ID Gene™ Bluetongue genotype 8 Duplex

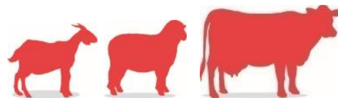
Ref: IDBTV8-50 / IDBTV8-100

50 / 100 tests

Real-time RT-PCR assay for **the qualitative** detection of the **Bluetongue virus genotype 8**

Suitable samples: ruminant whole blood

***In-vitro* Use**



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General information

- **Characteristics**

ID Gene™ Bluetongue genotype 8 Duplex (IDBTV8) kit is a real-time RT-PCR kit that amplifies a target sequence of Bluetongue viral genome, specific of genotype 8 (BTV8).

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

The kit contains a target positive control (TPC-BTV8) which is to be extracted in the same conditions as the samples to validate the extraction and amplification of the target.

This kit can be used to test ruminant whole blood collected in EDTA.

- **Kit composition and storage conditions**

The IDBTV8 kit contains the reagents shown below:

Reference	*Component*	*Volume*	*Description*
TPC-BTV8	Target Positive control	550 µl 1 vial	Inactivated BTV8, diluted in a virus-negative whole blood matrix, freeze-dried and calibrated at 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-BTV8	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mixture containing the reverse transcriptase, Taq polymerase and oligonucleotides for the amplification and detection of BTV8 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) in order to avoid multiple freeze/thaw cycles (> 3 cycles 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 **support.genetics@id-vet.com** regarding suitability with other thermal cyclers

- Heating block (ex : Thermomixer) capable of heating to 95°C

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 adhesive films or caps
- Refrigerated rack

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 IDBTV8 kit contains the following positive controls:

- Target Positive Control (TPC-BTV8):

This control consists of inactivated BTV8, diluted in a virus-negative whole blood matrix, calibrated at between 10 and 100 times the method detection limit (MDL).
This control is prepared and extracted in the same way as samples.
To validate the efficiency of the extraction and amplification processes.

- **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 as to quality 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 RNA. 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-BTV8) and 5 µl of Nuclease-free water. It is included in each run to control for the presence of aerosol contaminants.

Analysis procedure

▪ Extraction of viral RNA

The viral RNA must be extracted from the sample before being amplified by RT-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

▪ Extraction of the controls

The volumes of the control to extract are described in the table below:

Important:

- The volumes indicated are valid regardless of the extraction system.
- The controls must be extracted at the same time as the samples.

Control	***Volume***
TPC-BTV8	**50 µl**

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

▪ Preparation of the real-time PCR (RT-PCR) amplification reaction

1. Prepare an experimental plan for the analysis of the samples and controls, being sure to distance the positive control (TPC-BTV8) from the other samples.
2. Thaw the IDBTV8 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-BTV8** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-BTV8** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Heat extemporaneously the RNA extracted from samples and controls during **3 minutes (+1min) at 95°C (±2°C),** in order to denature the BTV8 double stranded RNA double.
6. **Immediately** after heating, place the denaturated extracts on ice or at 5°C (±3°C).
7. Add to reactional mix :
   - 5 µl of RNA extracted from each sample to be analyzed
   - 5 µl of RNA extracted from the TPC-BTV8
   - 5 µl of extracted NEC
   - 5 µl of Nuclease-free water (NAC)

Note: It is recommended to use a refrigerated rack during the sample distribution in order to keep the denatured state of the double stranded RNA.

8. 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****
BTV8	FAM	non fluorescent*
NTPCen	VIC/HEX	non fluorescent* (compatible VIC/HEX)

Note: For devices requiring an internal reference for optical calibration, the amplification mix IDBTV8 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 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) RNA 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 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-BTV8	Detected in FAM and VIC/HEX	Refer to the Cq value given in the quality control certificate
NTPCen	Detected in VIC/HEX in each sample	Presence of a characteristic curve
NEC	Nothing detected if water used Detected in VIC/HEX if negative sample used	Absence of a characteristic curve Presence of a characteristic curve
NAC	No detection	Absence of a characteristic curve

Note: TPC-BTV8 may be used to monitor variations in analytical sensitivity as a "sentinel" as it is calibrated at the limit of detection method.

Suggested interpretation of results

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

Sample	*BTV8 signal*	*NTPCen signal*	*Interpretation*
Individual	Detected	Detected or not detected	Animal detected as positive for BTV8
	Not detected	Detected	Animal **not** detected as positive for BTV8
	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 BTV8, 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 RNA 10 times in Nuclease-free water.
2. Repeat the amplification step 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: **info@id-vet.com**

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