INSTRUCTIONS FOR USE

MEAT-ID

Kit for multiplex detection of 16 animal species by real-time PCR (RT-PCR)

1. PRODUCT AND KIT COMPONENTS

Product	Commercial Reference	Number of reactions
MEAT-ID	AB0025	Σ 32

2. INTENDED PURPOSE

Intended purpose	MEAT-ID kit allows a rapid, easy, and reliable qualitative detection and identification of animal DNA in meat preparations, and other manufactured food products containing animal meat. Extracted DNA is used as starting material, and detection made using real-time PCR technology (RT-qPCR)
Test principle	MEAT-ID kit provides 3 different ready-to-use multiplex PCR mixes allowing the simultaneous detection of up to 16 animal species (**Mix-1**, 6 species; **Mix-2**, 5 species; **Mix-3**, 5 species). Please refer to table below. Detection is based on the amplification of a selected genomic region of the mitochondrial DNA (mtDNA), employing highly specific primers and TaqMan® probes, associated to FAM, HEX, ROX, ATTO 647N and ATTO 680 dyes. **Mix-3** is used also as 'endogenous meat control' mix. It contains a set of primers and probe (with FAM dye) able to amplify any DNA extracted from food preparations of animal-meat origin. The use of Mix-3 will assess the quality of DNA extraction and the lack of PCR inhibition due to sample material and extraction protocols. Notably, the meat control amplification will be absent in the case of vegan food preparations.
Intended users	Qualified laboratory staff trained in good laboratory practice and molecular diagnostic techniques, in particular real-time PCR.

Environment of use	Molecular biology laboratories with a structure and organization that complies with good laboratory practice (GLP) guidelines.
Required sample	Extracted DNA from fresh or frozen animal meat samples or other food preparations containing animal meat (please see 'Kit Usage' section for further information).
Analysis type	Qualitative

LIST OF DETECTABLE SPECIES

Target Species	English name	Mix	Fluorophore
Bos taurus	Cattle	1	FAM
Gallus gallus	Chicken	1	HEX
Ovis aries	Sheep	1	ROX
Sus scrofa domesticus	Pork	1	ATTO 647N *
Sus scrofa	Wild Boar		
Oryctolagus cuniculus	Rabbit	1	ATTO 680 *
Bos bison	Bison	2	FAM
Meleagris gallopavo	Turkey	2	HEX
Capra hircus	Goat	2	ROX
Equus caballus	Horse	2	ATTO 647N *
Lepus europaeus	Hare	2	ATTO 680 *
"All species"	Meat Control	3	FAM
Anas platyrhyncos	Mallard Duck	3	HEX
Capreolus capreolus	Roe Deer	3	ROX
Camelus bactrianus	Camel	3	ATTO 647N *
Camelus dromedarius	Dromedary		
Macropus (Osphranter) rufus	Kangaroo	3	ATTO 680 *

** ATTO 647N and ATTO 680 fluorophores can be detected in the CY5 and CY5.5 channels, respectively.*

3. TECHNICAL SPECIFICATIONS

Validated Sampling/transport medium	Use only material containing animal meat or food preparation that has been properly handled and stored prior to DNA extraction to avoid sample cross-contamination and degradation. Please follow GLP guidelines for molecular testing.
Recommended extraction method	Any available commercial kits compatible with DNA extraction from meat or food preparation. IMPORTANT: the method must be suitable for mitochondrial DNA (mtDNA) extraction.

<table>
<tr>
<td>Recommended instruments</td>
<td>
The use of MEAT-ID kit is potentially compatible with any real-time PCR thermocyclers equipped with FAM, HEX, ROX, ATTO 647N / CY5, ATTO 680 / CY5.5 fluorophores detectors.

The kit has been extensively tested using PCR machines equipped with 5 fluorescent channels.
<table>
<tr><th>Instrument name</th><th>Software</th><th>Manufacturer</th></tr>
<tr><td>CFX Opus 96 Real-Time PCR</td><td>CFX Maestro Dx SE version 2.0</td><td>Bio-Rad</td></tr>
<tr><td>QuantStudio 5</td><td>Design & Analysis 2 (DA2)</td><td>Thermo Fisher Scientific - Applied Biosystems</td></tr>
</table>
Other PCR instruments could be used. However, animal species identification will not include those targets detected by missing fluorescence channels, if any. For the “endogenous meat control” verification, FAM channel should always be present.

Please contact Appolon Bioteck for inquiries on compatibility of other real-Time PCR thermocyclers.
</td>
</tr>
</table>

4. CONTENT OF THE KIT

List of kit reagents:

Reagent	Part Number P/N	Cap color	Content	Quantity	Volume
Mix-1	Meat Mix-1	Transparent	Reaction mix: - Specific primers and probes for detection of Cattle, Chicken, Sheep, Pork or Wild boar, Rabbit. - Polymerase Master Mix - Molecular biology grade water	1 tube	700 µL
Mix-2	Meat Mix-2	Yellow	Reaction mix: - Specific primers and probes for detection of Bison, Turkey, Goat, Horse, Hare. - Polymerase Master Mix - Molecular biology grade water	1 tube	700 µL

Reagent	Part Number P/N	Cap color	Content	Quantity	Volume
Mix-3	Meat Mix-3	Purple	Reaction mix: - Specific primers and probes for detection of the Meat Control, Mallard Duck, Roe Deer, Camel or Dromedary, Kangaroo. - Polymerase Master Mix - Molecular biology grade water	1 tube	700 µL

Description of reagents and limitations
The substances in this kit are not classified as dangerous substances under the European Regulation (EC) 1272/2008 and the Occupational Safety and Health Standards, Hazard Communication, Toxic and Hazard Substances (March, 21th 2017) (29 C.F.R., pt. 1910, subpt. Z).

5. TRANSPORT, STORAGE AND STABILITY

Steps	Conditions details
Transport	- Transport of the product must comply with current regulations in force and temperature maintained below -15°C.
Storage / Conservation	- Reagents should be stored in a dark, dry place. - Storage temperatures must be between -25°C and -15°C. - Do not exceed 5 thaw/freeze cycles.
Shelf life	- Please refer to the expiry date on the product label.

6. REQUIRED EQUIPMENTS AND ACCESSORIES NOT SUPPLIED WITH THE KIT

Steps	Equipment type
Laboratory Equipment	Freezer at -20°C, Refrigerator at +4°C
	Powder-free disposable gloves
	Laboratory coat
	Benchtop centrifuge with plate and/or for 1.5 mL and 2.0 mL tubes
	Benchtop vortexer
	Adjustable micropipettes P10 – P20 or P100
	Appropriate sterile filter cones
	1.5 mL Eppendorf microtubes or equivalent
	Microtubes, strips or 96-position PCR plates 0.2 mL
	PCR-specific optical films (or caps for strips)
	Cold rack or crushed ice

Steps	Equipment type
Extraction	Any kit compatible with DNA extraction from meat or food preparation. IMPORTANT: the method must be suitable for mitochondrial DNA (mtDNA) extraction.
PCR Amplification and analysis	Preferred: Thermocycler CFX Opus 96 Real-Time PCR associated to Bio-Rad CFX Maestro software (Bio-Rad). Thermo Fisher Scientific - Applied Biosystems QuantStudio 5 associated to Design & Analysis 2 (DA2) software. Or any other real-time PCR compatible instruments with dedicated software.
Other reagents	Molecular biology grade water
	DNA-RNA decontamination products

7. SAMPLING PROCEDURES

Samples of meat or other food preparation should be processed immediately upon collection, kept at +4°C for short-term storage or kept at -20°C for long-term storage.

Please follow good laboratory practice (GLP) guidelines for molecular biology and strongly avoid cross-contamination between collected samples.

8. USE PROTOCOL

8.1 NUCLEIC ACID EXTRACTION

- It is recommended to follow the instructions for use of the kits and extraction systems selected.
- Control of DNA purity and quantity after extraction: we recommend a UV spectrophotometer measurement giving an absorbance ratio A260/A280 between 1.7 and 1.9. We also recommend the use of a fluorescence-based instrument for quantification, such as **Qubit™** Fluorometer. Optimal DNA sample concentration should be between 0.01 ng/µL and 1 ng/µL. Please refer to 'Kit Sensitivity' section for more detailed information.

8.2 REAL-TIME AMPLIFICATION

IMPORTANT REMARKS

- For each sample, all 3 PCR mixes must be used.
- The PCR products generated during the assay must be considered as contamination sources for PCR. Therefore, observe the standard guidelines for working in a PCR molecular biology laboratory to prevent contaminations.

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PROTOCOL

1. Thaw all provided reagents on ice and check for required mix volumes, considering:
   - 1 reaction per mix per extracted DNA sample to be tested.
   - 1 reaction “No Template Control” (NTC) per mix, as contamination test.
2. Gently vortex all kit tubes and extracted DNA samples.

3. Centrifuge all kit tubes and DNA samples to reduce risk of aerosol.

4. Distribute the reaction mixes according to the experiment design:
   a. For each sample, dispense 20 µL of the 3 PCR mixes into 3 separate plate wells.
   b. For the NTC, dispense 20 µL of the 3 PCR mixes into 3 separate plate wells.
   c. Add 5 µL of extracted DNA to each reaction mix (Sample Test, **Table 1**).
   d. Add 5 µL of molecular biology grade water to the dedicated NTC reaction mix wells (NTC Test, **Table 2**).

Table 1: Volumes to be distributed for sample test.

SAMPLE TEST	Mix-1	Mix-2	Mix-3
Kit Reagent	20 µL	20 µL	20 µL
Extracted DNA Sample	5 µL	5 µL	5 µL
Total reaction volume	**25 µL**	**25 µL**	**25 µL**

Table 2: Volumes to be distributed for NTC test.

NTC TEST	Mix-1	Mix-2	Mix-3
Kit Reagent	20 µL	20 µL	20 µL
Water (ultra-pure, molecular biology grade)	5 µL	5 µL	5 µL
Total reaction volume	**25 µL**	**25 µL**	**25 µL**

5. Seal the plate with compatible optical adhesive film and centrifuge it.

6. Place the plate in the thermocycler in the correct position.

7. Prepare the run setup using the software of choice, including all the necessary information for traceability, the PCR amplification protocol (**Table 3**) and plate design. Make sure to activate detection of FAM, HEX, ROX, ATTO647N/CY5 and ATTO680/CY5.5 channels on the thermocycler. In the plate design, please assign, for each of the 3 PCR mixes, the correct target names to the corresponding dyes (**Table 4**) and specify desired sample names. Please refer to **Table 5** for an example of plate design.

8. Start the PCR run.

Table 3: PCR amplification protocol

Steps	Process	Program		Cycles	Fluorescence Acquisition
		Duration	Temperature		
1	*Taq* polymerase activation	2 min	95°C	1	None
2	DNA denaturation	10 sec	95°C	35	None
3	Annealing / Extension	5-20 sec *	60°C		Yes

(*) PCR annealing/extension step might vary depending on the thermocycler. Please contact Appolon Bioteck support team if assistance for any kind of assay optimization is needed.

Table 4: Multiplex PCR dyes and target assignment.

Mix used in the well	Fluorophore	Target
Mix-1	FAM	Cattle
	HEX	Chicken
	ROX	Sheep
	ATTO 647N / CY5	Pork / Wild Boar
	ATTO 680 / CY5.5	Rabbit
Mix-2	FAM	Bison
	HEX	Turkey
	ROX	Goat
	ATTO 647N / CY5	Horse
	ATTO 680 / CY5.5	Hare
Mix-3	FAM	Meat Control
	HEX	Mallard Duck
	ROX	Roe Deer
	ATTO 647N / CY5	Camel / Dromedary
	ATTO 680 / CY5.5	Kangaroo

Table 5: Example of plate design.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Mix-1 NTC	Mix-2 NTC	Mix-3 NTC									
B	**Mix-1** Sample **#01**	**Mix-2** Sample **#01**	**Mix-3** Sample **#01**									
C	**Mix-1** Sample **#02**	**Mix-2** Sample **#02**	**Mix-3** Sample **#02**									
D	**Mix-1** Sample **#03**	**Mix-2** Sample **#03**	**Mix-3** Sample **#03**									
E	**Mix-1** Sample **#04**	**Mix-2** Sample **#04**	**Mix-3** Sample **#04**									
F	**Mix-1** Sample **#05**	**Mix-2** Sample **#05**	**Mix-3** Sample **#05**									
G	**Mix-1** Sample **#06**	**Mix-2** Sample **#06**	**Mix-3** Sample **#06**									
H	**Mix-1** Sample **#07**	**Mix-2** Sample **#07**	**Mix-3** Sample **#07**									

9. RESULTS ANALYSIS AND INTERPRETATION

IMPORTANT REMARKS

MEAT-ID kit generates qualitative results indicating the 'presence' or 'absence' of a specific target in the sample material used for DNA extraction and PCR amplification.

Definition of "Target": selected genomic region of the mitochondrial DNA (mtDNA) of a given animal species.

'Presence' or 'absence' is defined by the assay specific detection range and limits (please refer to "Kit Specificity" and "Kit Sensitivity" sections). Different PCR signal intensities can be observed using the assay. Relative fluorescent signal is generally correlated with the amount of target copies in the starting material. However, MEAT-ID kit has not been validated as tools for absolute quantification.

As general guideline for data interpretation, we suggest the following:

SPECIES DETECTED: **15 < Cq < 32** (presence in the PCR reaction of mtDNA, from ≥ 500 pg to ~ 5 pg).

SPECIES IN TRACE: **32 < Cq < 35** (presence in PCR reaction of mtDNA ≤ 5 pg).

SPECIES ABSENT: **Cq > 35** (mtDNA absent in PCR reaction or quantity below the limit of detection).

METHOD OF ANALYSIS

Detection of animal species is based on the analysis of multiplex PCR amplification curves (Relative fluorescence units – RFU vs. PCR cycles plots) associated to the 5 different dye channels.

'Presence' of a given animal species is dictated by 2 simultaneous PCR amplification (**Table 6**):

1) Species-specific amplification (Mix-1 or Mix-2 or Mix-3, any of the 5 dyes).
2) Meat Control amplification (Mix-3, FAM dye).

IMPORTANT: no amplification signal should be detected in the NTC test reactions.

Table 6: Animal species detection.

Animal Species	Species-Specific Amplification	Meat Control Amplification
Cattle	Mix-1 – FAM	Mix 3 – FAM
Chicken	Mix-1 – HEX	Mix 3 – FAM
Sheep	Mix-1 – ROX	Mix 3 – FAM
Pork	Mix-1 – ATTO 647N / CY5	Mix 3 – FAM
Wild Boar	Mix-1 – ATTO 647N / CY5	Mix 3 – FAM
Rabbit	Mix-1 – ATTO 680 / CY5.5	Mix 3 – FAM
Bison	Mix-2 – FAM	Mix 3 – FAM
Turkey	Mix-2 – HEX	Mix 3 – FAM
Goat	Mix-2 – ROX	Mix 3 – FAM
Horse	Mix-2 – ATTO 647N / CY5	Mix 3 – FAM
Hare	Mix-2 – ATTO 680 / CY5.5	Mix 3 – FAM
Mallard Duck	Mix-3 – HEX	Mix 3 – FAM
Roe Deer	Mix-3 – ROX	Mix 3 – FAM
Camel (Bactrian)	Mix-3 – ATTO 647N / CY5	Mix 3 – FAM
Dromedary	Mix-3 – ATTO 647N / CY5	Mix 3 – FAM
Kangaroo	Mix-3 – ATTO 680 / CY5.5	Mix 3 – FAM

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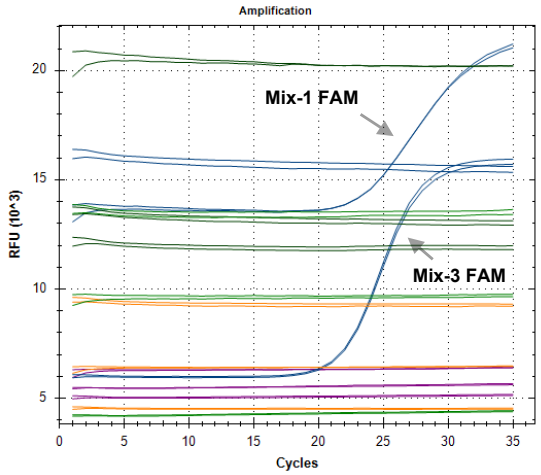
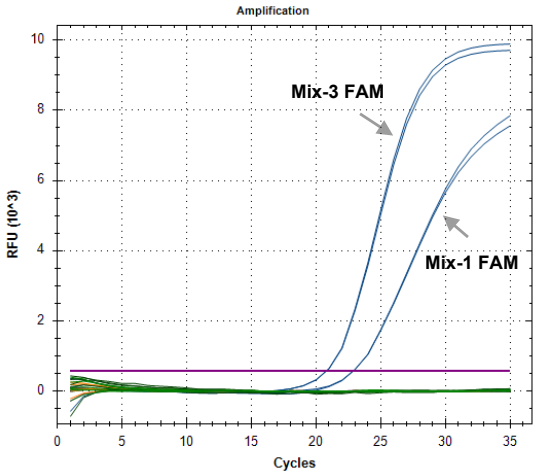
TEST EXAMPLE #1: **CATTLE**

Raw Fluorescence Data



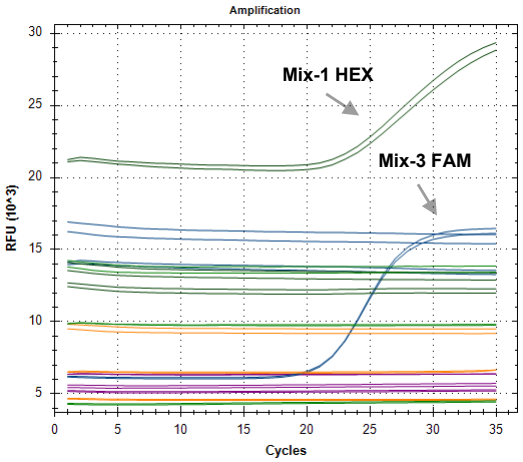
Processed Fluorescence Data



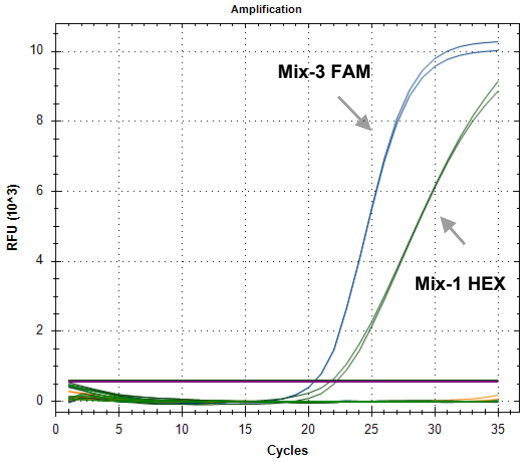
	FAM	HEX	ROX	ATTO647N / CY5	ATTO680 / CY5.5
Mix-1	**Amplification** ***(Cattle)***	No Amplification	No Amplification	No Amplification	No Amplification
Mix-2	No Amplification	No Amplification	No Amplification	No Amplification	No Amplification
Mix-3	**Amplification** ***(Meat Control)***	No Amplification	No Amplification	No Amplification	No Amplification

TEST EXAMPLE #2: **CHICKEN**

Raw Fluorescence Data



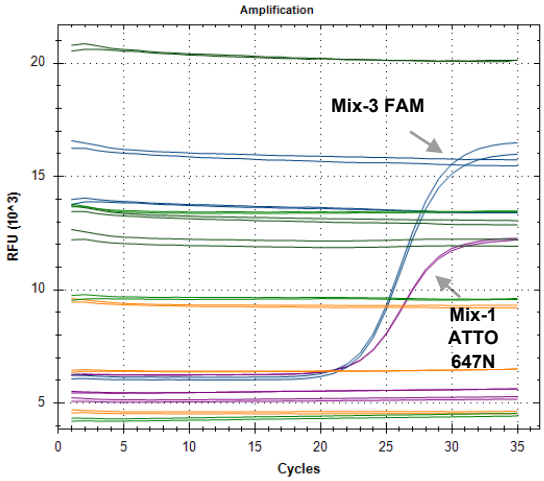
Processed Fluorescence Data



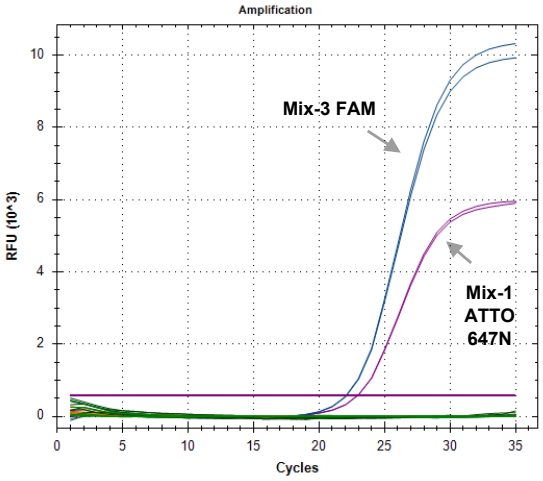
	FAM	HEX	ROX	ATTO647N / CY5	ATTO680 / CY5.5
Mix-1	No Amplification	**Amplification** ***(Chicken)***	No Amplification	No Amplification	No Amplification
Mix-2	No Amplification	No Amplification	No Amplification	No Amplification	No Amplification
Mix-3	**Amplification** ***(Meat Control)***	No Amplification	No Amplification	No Amplification	No Amplification

TEST EXAMPLE #3: **PORK**

Raw Fluorescence Data



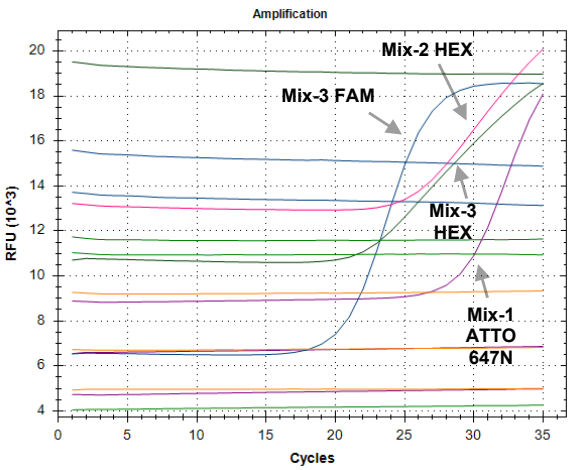
Processed Fluorescence Data



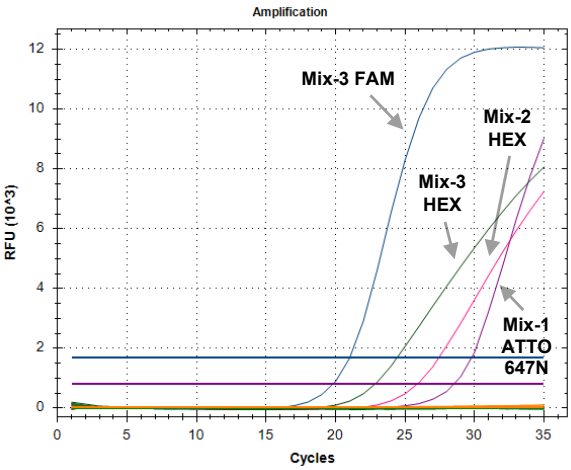
	FAM	HEX	ROX	ATTO647N / CY5	ATTO680 / CY5.5
Mix-1	No Amplification	No Amplification	No Amplification	Amplification *(Pork / Wild Boar)*	No Amplification
Mix-2	No Amplification	No Amplification	No Amplification	No Amplification	No Amplification
Mix-3	Amplification *(Meat Control)*	No Amplification	No Amplification	No Amplification	No Amplification

TEST EXAMPLE #4: **MALLARD DUCK + TURKEY + PORK / WILD BOAR**

Raw Fluorescence Data



Processed Fluorescence Data



	FAM	HEX	ROX	ATTO647N / CY5	ATTO680 / CY5.5
Mix-1	No Amplification	No Amplification	No Amplification	Amplification *(Pork / Wild boar)*	No Amplification
Mix-2	No Amplification	Amplification *(Turkey)*	No Amplification	No Amplification	No Amplification
Mix-3	Amplification *(Meat Control)*	Amplification *(Mallar Duck)*	No Amplification	No Amplification	No Amplification

10. KIT SPECIFICITY

Primer and probe sequences used in this assay have been carefully chosen after *in silico* analyses and *in vitro* testing.

The table below shows the results (mean of Cq values) from testing of 16 animal species, after DNA extraction from a sample of lean skeletal muscle tissue. Before PCR analysis, the concentration of the DNA samples was normalized along the species to a fixed value of 0.05 ng/µL (**Table 7**). No evidence for cross reactivities have been found.

Table 7: Highly specific animal species detection by using MEAT-ID kit.

Meat Sample	MEAT-ID Mix	FAM	HEX	ROX	ATTO 647N / CY5	ATTO 680 / CY5.5
Cattle	Mix-1	**22.85**	ND	ND	ND	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**20.76**	ND	ND	ND	ND
Chicken	Mix-1	ND	**21.98**	ND	ND	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**20.43**	ND	ND	ND	ND
Sheep	Mix-1	ND	ND	**22.22**	ND	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**21.12**	ND	ND	ND	ND
Pork	Mix-1	ND	ND	ND	**22.90**	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**22.05**	ND	ND	ND	ND
Wild Boar	Mix-1	ND	ND	ND	**20.52**	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**20.20**	ND	ND	ND	ND
Rabbit	Mix-1	ND	ND	ND	ND	**22.23**
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**21.06**	ND	ND	ND	ND
Bison	Mix-1	25.06	ND	ND	ND	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**21.95**	ND	ND	ND	ND
Turkey	Mix-1	ND	**23.12**	ND	ND	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**22.07**	ND	ND	ND	ND
Goat	Mix-1	ND	ND	**22.26**	ND	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**20.67**	ND	ND	ND	ND
Horse	Mix-1	ND	ND	ND	**23.15**	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**21.84**	ND	ND	ND	ND

Meat Sample	MEAT-ID Mix	FAM	HEX	ROX	ATTO 647N / CY5	ATTO 680 / CY5.5
Hare	Mix-1	ND	ND	ND	ND	**21.39**
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**19.44**	ND	ND	ND	ND
Mallard Duck	Mix-1	ND	**21.19**	ND	ND	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**19.78**	ND	ND	ND	ND
Roe Deer	Mix-1	ND	ND	**21.16**	ND	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**19.21**	ND	ND	ND	ND
Camel	Mix-1	ND	ND	ND	**21.21**	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**20.39**	ND	ND	ND	ND
Dromedary	Mix-1	ND	ND	ND	**23.44**	ND
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**21.78**	ND	ND	ND	ND
Kangaroo	Mix-1	ND	ND	ND	ND	**23.96**
	Mix-2	ND	ND	ND	ND	ND
	Mix-3	**20.40**	ND	ND	ND	ND

11. KIT SENSITIVITY

The sensitivity of the assay is mainly determined by the number of PCR cycles. Sensitivity can be modulated adjusting the PCR cycles number, according to the experimental demands. However, to reach the maximal sensitivity of a specific target amplification, we recommend not to run more than 35 PCR cycles.

The assay sensitivity has been measured with 2 different methods (with 35 PCR cycles):

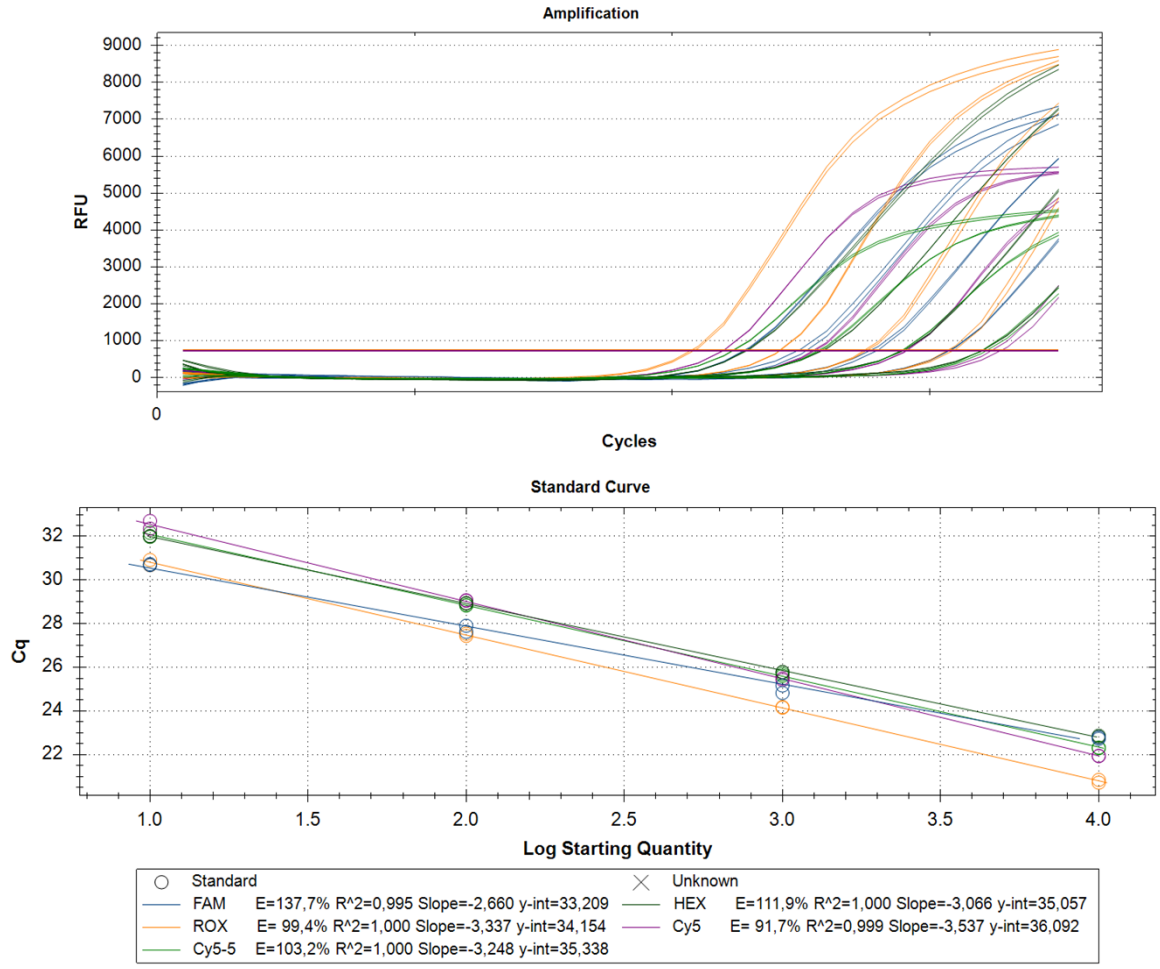
- with serial dilutions of normalized DNA extracts from fresh or frozen animal skeletal muscle tissue samples, covering a concentration range from **100 pg/µL** to **0.1 pg/µl**.
- with serial dilutions of synthetic dsDNA mapping the targets' amplicons, covering a concentration range from **1E+10 copies/mL** to **1E+03 copies/mL**.

DNA Extracts								
Concentration (**pg/µL**)	100 pg/mL		10 pg/mL		1 pg/mL		0.1 pg/mL	
Quantity / reaction (**pg**)	500 pg		50 pg		5 pg		0.5 pg	
Synthetic dsDNA								
Concentration (**cp/mL**)	1E+10	1E+09	1E+08	1E+07	1E+06	1E+05	1E+04	1E+3
Quantity / reaction (**cp**)	5E+7	5E+6	5E+5	5E+4	5E+3	5E+2	5E+1	5E+0

TEST ON DNA EXTRACT FROM MEAT SAMPLE

Results from **Mix-1** testing are shown in the figures below, as an example.

- **FAM** (blue): CATTLE
- **HEX** (green): CHICKEN
- **ROX** (yellow): SHEEP
- **ATTO 647N** (purple): DROMEDARY
- **ATTO 680** (light green): RABBIT



Results from the sensitivity test on DNA extracts are summarized in **Table 8** and **Table 9**.

The “Limit of Detection” (**LOD**) of MEAT-ID kit has been set to **0.1 pg/µL** as minimum DNA concentration detectable, corresponding to **0.5 pg per reaction**.

IMPORTANT: the sensitivity values given in **Table 8** are valid for amplifications of individual targets. In mixtures of multiple target sequences, the detection limit of targets may vary depending on the target amplification efficiency. Also, the detection limit of targets with lower concentration will be raised due to the competition with targets of higher concentration for PCR reagents in the mixture.

Table 8: Results from the sensitivity test on DNA extracts.

DNA Sample	Target	MEAT-ID Mix	Fluorophore	Quantity	Cq value (mean)
Cattle	CATTLE	1	FAM	100 pg/µL	22.76
				10 pg/µL	25.00
				1 pg/µL	27.77
				0.1 pg/µL	30.71
Chicken	CHICKEN	1	HEX	100 pg/µL	22.84
				10 pg/µL	25.78
				1 pg/µL	28.94
				0.1 pg/µL	32.01
Sheep	SHEEP	1	ROX	100 pg/µL	20.78
				10 pg/µL	24.17
				1 pg/µL	27.49
				0.1 pg/µL	30.80
Pork	PORK / WILD BOAR	1	ATTO 647N / CY5	100 pg/µL	21.94
				10 pg/µL	25.46
				1 pg/µL	29.06
				0.1 pg/µL	32.53
Rabbit	RABBIT	1	ATTO 680 / CY5.5	100 pg/µL	22.31
				10 pg/µL	25.65
				1 pg/µL	28.84
				0.1 pg/µL	32.07
Bison	BISON	2	FAM	100 pg/µL	23.48
				10 pg/µL	26.66
				1 pg/µL	30.31
				0.1 pg/µL	33.36
Turkey	TURKEY	2	HEX	100 pg/µL	22.75
				10 pg/µL	26.10
				1 pg/µL	29.43
				0.1 pg/µL	33.18
Goat	GOAT	2	ROX	100 pg/µL	21.73
				10 pg/µL	25.11
				1 pg/µL	28.56
				0.1 pg/µL	32.10
Horse	HORSE	2	ATTO 647N / CY5	100 pg/µL	22.39
				10 pg/µL	25.85
				1 pg/µL	29.29
				0.1 pg/µL	32.93
Hare	HARE	2	ATTO 680 / CY5.5	100 pg/µL	22.10
				10 pg/µL	25.42
				1 pg/µL	29.25
				0.1 pg/µL	33.34
Mallard Duck	MALLARD DUCK	3	HEX	100 pg/µL	21.25
				10 pg/µL	24.55
				1 pg/µL	27.91
				0.1 pg/µL	31.62
Roe Deer	ROE DEER	3	ROX	100 pg/µL	20.16
				10 pg/µL	23.40
				1 pg/µL	26.82
				0.1 pg/µL	30.48
Dromedary	CAMEL / DROMEDARY	3	ATTO 647N / CY5	100 pg/µL	22.53
				10 pg/µL	26.20
				1 pg/µL	29.70
				0.1 pg/µL	32.50
Kangaroo	KANGAROO	3	ATTO 680 / CY5.5	100 pg/µL	22.30
				10 pg/µL	25.35
				1 pg/µL	28.79
				0.1 pg/µL	32.33

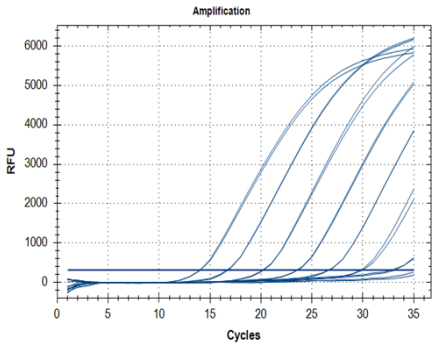
Table 9: PCR efficiency and linearity on DNA extracts

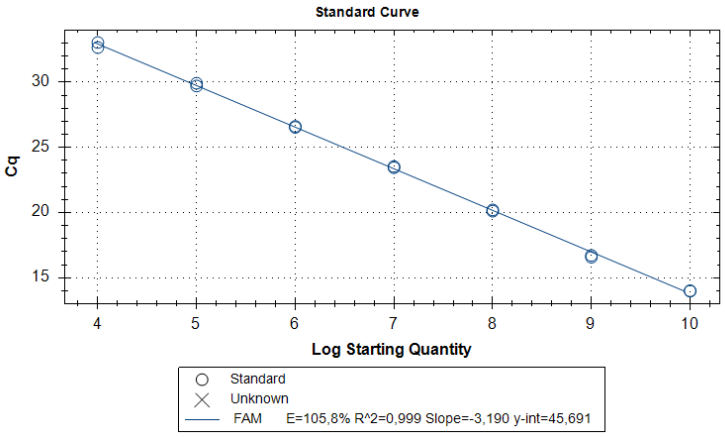
MEAT-ID Mix	Target	Fluorophore	Efficiency %	Slope	Y-Intercept	R^2
1	CATTLE	FAM	137,68	-2,660	33,209	0,995
	CHICKEN	HEX	111,91	-3,066	35,057	1,000
	SHEEP	ROX	99,36	-3,337	34,154	1,000
	PORK	ATTO 647N / CY5	91,74	-3,537	36,092	0,999
	RABBIT	ATTO 680 / CY5.5	103,20	-3,248	35,338	1,000
2	BISON	FAM	99,82	-3,326	36,770	0,997
	TURKEY	HEX	94,4	-3,465	36,527	0,999
	GOAT	ROX	92,83	-3,507	35,686	1,000
	HORSE	ATTO 647N / CY5	92,90	-3,505	36,379	0,999
	HARE	ATTO 680 / CY5.5	84,10	-3,773	36,965	0,998
3	MALLARD DUCK	HEX	95,1	-3,446	34,947	0,999
	ROE DEER	ROX	95,4	-3,438	33,81	0,999
	DROMEDARY	ATTO 647N / CY5	99,18	-3,342	36,090	0,996
	KANGAROO	ATTO 680 / CY5.5	98,7	-3,354	35,575	0,998

TEST ON SYNTHETIC dsDNA

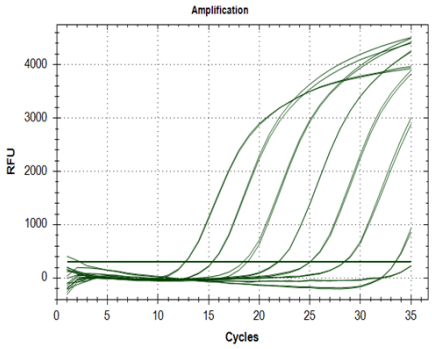
Results from **Mix-1** testing are shown in the figures below, as an example.

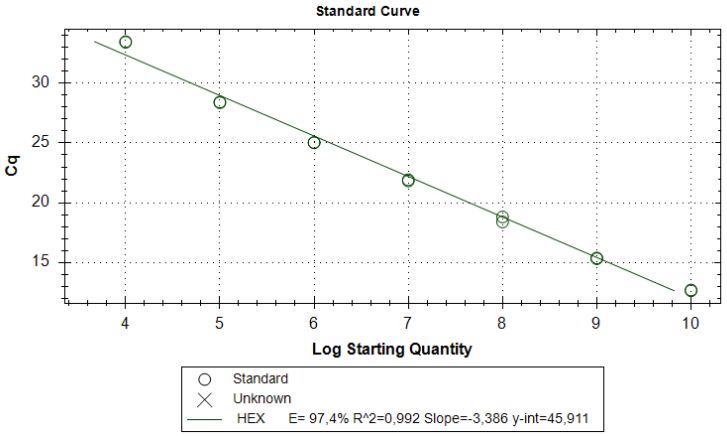
FAM: **CATTLE**



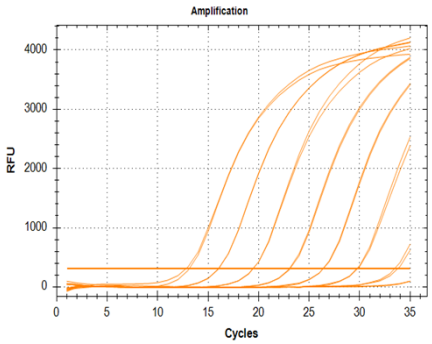


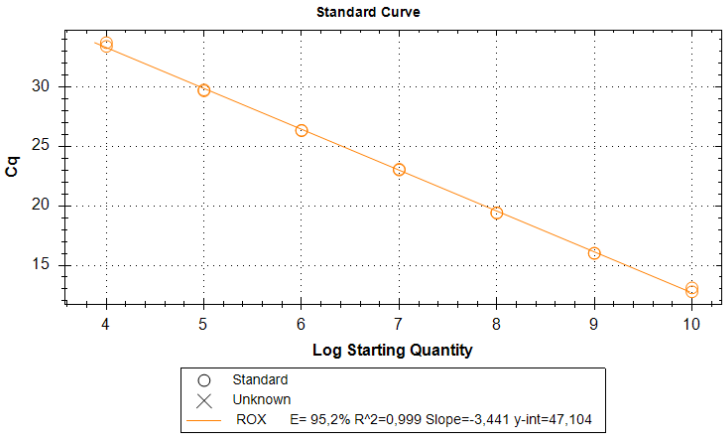
HEX: **CHICKEN**



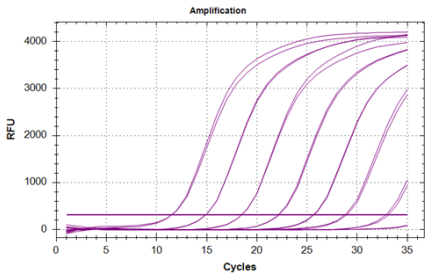


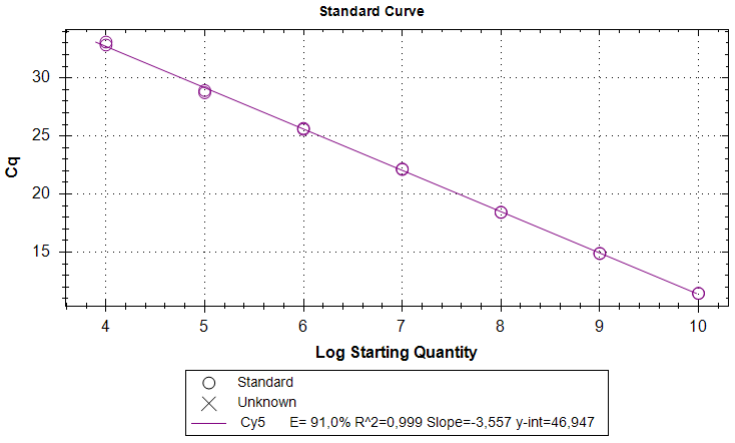
ROX: **SHEEP**



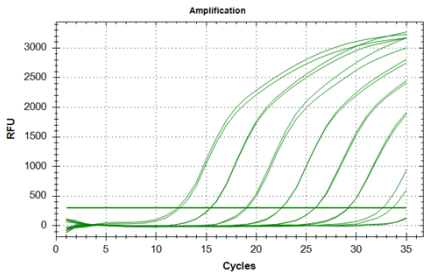


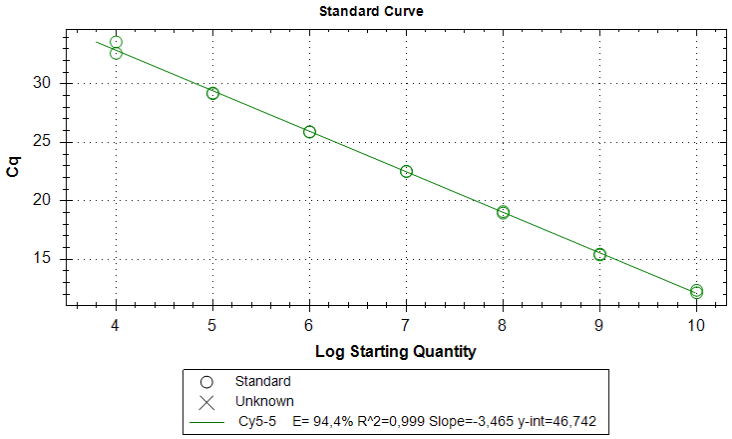
ATTO 647N: **DROMEDARY**





ATTO 680: **RABBIT**





Results from the sensitivity test on synthetic dsDNA are summarized in **Table 10** and **Table 11**.

The “Limit of Detection” (LOD) of MEAT-ID kit has been set to **1E+09 target copies/mL** (**Upper LOD**) and **1E+04 target copies/mL** (**Lower LOD**), corresponding to **5E+6** and **5E+1 copies per reaction**, respectively.

Table 10: Results from the sensitivity test on synthetic dsDNA.

Sample	Target	MEAT-ID Mix	Fluorophore	Quantity	Cq value (mean)
Cattle	CATTLE	MIX-1	FAM	1E+10	14.00
				1E+09	16.65
				1E+08	20.16
				1E+07	23.49
				1E+06	26.57
				1E+05	29.82
				1E+04	32.85
				1E+03	0.00
Chicken	CHICKEN	MIX-1	HEX	1E+10	12.68
				1E+09	15.36
				1E+08	18.62
				1E+07	21.88
				1E+06	25.04
				1E+05	28.40
				1E+04	33.46
				1E+03	0.00
Sheep	SHEEP	MIX-1	ROX	1E+10	12.94
				1E+09	16.01
				1E+08	19.41
				1E+07	23.06
				1E+06	26.35
				1E+05	29.73
				1E+04	33.60
				1E+03	0.00

Sample	Target	MEAT-ID Mix	Fluorophore	Quantity	Cq value (mean)
Pork	PORK / WILD BOAR	MIX-1	ATTO 647N / CY5	1E+10	11.45
				1E+09	14.89
				1E+08	18.43
				1E+07	22.15
				1E+06	25.62
				1E+05	28.82
				1E+04	32.97
				1E+03	0.00
Rabbit	RABBIT	MIX-1	ATTO 680 / CY5.5	1E+10	12.25
				1E+09	15.41
				1E+08	19.02
				1E+07	22.52
				1E+06	25.90
				1E+05	29.20
				1E+04	33.11
				1E+03	0.00
Bison	BISON	MIX-2	FAM	1E+10	13.42
				1E+09	16.80
				1E+08	20.11
				1E+07	24.49
				1E+06	28.85
				1E+05	30.15
				1E+04	34.65
				1E+03	0.00
Turkey	TURKEY	MIX-2	HEX	1E+10	11.42
				1E+09	14.58
				1E+08	18.07
				1E+07	21.41
				1E+06	24.98
				1E+05	28.30
				1E+04	31.60
				1E+03	0.00
Goat	GOAT	MIX-2	ROX	1E+10	12.35
				1E+09	15.45
				1E+08	18.94
				1E+07	22.35
				1E+06	25.89
				1E+05	29.19
				1E+04	32.36
				1E+03	0.00
Horse	HORSE	MIX-2	ATTO 647N / CY5	1E+10	11.31
				1E+09	14.52
				1E+08	17.98
				1E+07	21.49
				1E+06	25.07
				1E+05	28.58
				1E+04	32.14
				1E+03	0.00
Hare	HARE	MIX-2	ATTO 680 / CY5.5	1E+10	12.99
				1E+09	16.06
				1E+08	19.47
				1E+07	23.07
				1E+06	26.58
				1E+05	30.26
				1E+04	33.67
				1E+03	0.00

Sample	Target	MEAT-ID Mix	Fluorophore	Quantity	Cq value (mean)
Mallard Duck	MALLARD DUCK	MIX-3	HEX	1E+10	11.67
				1E+09	14.94
				1E+08	18.45
				1E+07	21.90
				1E+06	25.29
				1E+05	28.56
				1E+04	32.12
				1E+03	0.00
Roe Deer	ROE DEER	MIX-3	ROX	1E+10	12.48
				1E+09	15.99
				1E+08	19.42
				1E+07	23.02
				1E+06	26.32
				1E+05	29.82
				1E+04	33.19
				1E+03	0.00
Camel	CAMEL / DROMEDARY	MIX-3	ATTO 647N / CY5	1E+10	12.11
				1E+09	15.46
				1E+08	19.06
				1E+07	22.52
				1E+06	25.89
				1E+05	29.21
				1E+04	32.74
				1E+03	0.00
Kangaroo	KANGAROO	MIX-3	ATTO 680 / CY5.5	1E+10	12.24
				1E+09	15.96
				1E+08	19.66
				1E+07	23.26
				1E+06	26.77
				1E+05	30.33
				1E+04	34.07
				1E+03	0.00

Table 11: PCR efficiency and linearity on synthetic dsDNA.

MEAT-ID Mix	Target	Fluorophore	Efficiency %	Slope	Y-Intercept	R^2
1	CATTLE	FAM	105,82	-3,190	45,691	0,999
	CHICKEN	HEX	97,37	-3,386	45,911	0,992
	SHEEP	ROX	95,25	-3,441	47,104	0,999
	PORK	ATTO 647N / CY5	91,04	-3,557	46,947	0,999
	RABBIT	ATTO 680 / CY5.5	94,36	-3,465	46,742	0,999
2	BISON	FAM	91,63	-3,540	48,850	0,988
	TURKEY	HEX	97,24	-3,390	45,208	1,000
	GOAT	ROX	97,92	-3,373	45,970	1,000
	HORSE	ATTO 647N / CY5	93,46	-3,489	46,008	1,000
	HARE	ATTO 680 / CY5.5	93,63	-3,485	47,552	0,999
3	MALLARD DUCK	HEX	96,50	-3,409	45,709	1,000
	ROE DEER	ROX	94,79	-3,454	47,068	1,000
	DROMEDARY	ATTO 647N / CY5	95,44	-3,436	46,477	1,000
	KANGAROO	ATTO 680 / CY5.5	88,96	-3,618	48,515	1,000

12. TROUBLESHOOTING

AMPLIFICATION ISSUES

Possible causes	Solutions
Sub-optimal performance by the amplification mix	• Avoid more than 5 thaw/freeze cycles of the amplification mix. • Use a cold block (+4°C) when dispensing mix and samples. • The Taq enzymes are sensitive to temperature variations. Do not leave them at room temperature beyond the time required to handle the kit. • Check the expiration date of the batch used. • Follow the instructions for product transport, storage and stability.
Non-compliance with sample collection, transport, and storage conditions	• Follow the instructions for sample preparation, transport, and storage. • Check the time between sample collection, processing, and PCR analysis.
Problems during nucleic acid extraction	• Check that samples are homogenized before the extraction step. • Check the protocol, reagents and equipment used to extract samples. • Always carry out preventive maintenance on extraction equipment in accordance with the manufacturer's recommendations.
Reagent or sample distribution error	• Draw up a plate design to limit dispensing errors. • Check the calibration of pipettes, which should be subject to regular metrological control. • Ensure that mix and samples are well homogenized before their distribution to the PCR plate wells.
Thermocycler programming error	• Check that the thermocycler used is compatible with the one(s) validated with the kit. • Check all thermocycler programming parameters (detection channels and mode, number of cycles, temperature, time, reaction volume, etc.).
Amplification environment	• Check the Peltier block’s performance according to its manufacturer recommendations. • Perform a preventive maintenance on the PCR equipment according to manufacturer's recommendations. • Check that the PCR plate is properly sealed with the optical film. • Check that the plastic consumables used are those recommended for the instrument (semi-skirted plate, low-profile tubes/ plate, etc.).

AMPLIFICATION INHIBITION / ABSENCE

Possible causes	Solutions
Problem during nucleic acid extraction	• Check that samples are well homogenized before sampling for nucleic acid extraction. • Check all extraction equipment, protocols, and solutions. • Always carry out preventive maintenance on extraction equipment in accordance with the manufacturer's recommendations.
Presence of inhibitory substances in the meat/food matrix	• Repeat the PCR test using diluted DNA samples (*e.g.*, 1:10; 1:100)
Presence of material of animal origin (meat) in the food sample that is not detectable by the kit (species not listed)	• Check the list animal species that are detectable by MEAT-ID kit. • Check that the “Meat Control” (Mix-3 / FAM) is amplified acceptably.
Absence of material of animal origin (meat) in the food sample.	• Re-run the PCR adding a meat positive control sample, to verify the correct performance of the kit. • Call food sample with no amplification as 'vegan’.

CONTAMINATION ISSUES

Possible causes	Solutions
Meat / food matrix cross-contamination	• Repeat sampling and DNA extraction.
Kit reagents cross-contamination	• Discard reagent used tubes and use a new product.
Contamination during experimentation	• Decontaminate all small laboratory equipment with products suitable for removing nucleic acids. • Change products used (water, PCR reagent, etc.). • Repeat the experiment following carefully the protocol provided with the kit.
NTC Contamination	• Repeat the experiment. • Change the molecular biology grade water used.

13. PRECAUTIONS FOR USE AND LIMITATIONS

- *Kit pre-use precautions:*

- This kit should only be used by personnel trained in good laboratory practice and competent in molecular biology.
- Check the integrity of the kit before use: the packaging must be undamaged, and the kit must comply with specifications (tubes number, volume, etc.)
- Do not use the product if signs of contamination are present (presence of foreign bodies in tubes).
- Do not use the product if tampering is visible.

- Do not use the product after the indicated expiration date.
- Read all instructions carefully before use. Failure to do so may lead to incorrect results.
- Compliance with sample collection procedures (collection, transport, storage) is essential for optimum performance of this test, in order to avoid incorrect results.
- Do not use this kit with any type of sample or equipment other than those recommended in this manual, as this may lead to erroneous results.
- Nucleic acid extraction and amplification must be carried out using the equipment and methods listed in this leaflet. For any other equipment not listed in this manual, we recommend that its effectiveness be assessed by the laboratory's internal validation procedure.
- For equipment not supplied but to be used in combination with this kit, please refer to the product's instructions for use before use.

- *Precautions when using the kit:*

⚠ Caution: Please handle the tube containing the Mix in a dark place!

- Use all protective equipment when handling samples and kit reagents to avoid contamination.
- Use separate workstations for sample preparation / nucleic acid extraction and amplification reactions. Never introduce amplified product into the reagent preparation and/or nucleic acid
- extraction (sample preparation) area.
- Prepare samples under a microbiological safety station (MSS).
- Use only RNase- and DNase-free filter pipette tips.
- Use new tips for each pipetting step.
- Close kit component vials immediately after use and never interchange lids.
- Do not pool reagents from different batches or from different vials within the same batch.
- Do not substitute reagents between batches.
- Avoid skin contact with kit reagents. In case of skin contact, wash immediately with plenty of water.

The Safety Data Sheet is available on request from support@appolonbioteck.com

14. CONTRAINDICATIONS

To date, Appolon Bioteck has not identified any contraindications for this product.

15. ADVERSE EVENTS

Any serious incident involving the product is reported to Appolon Bioteck and to the competent health authority of the Member State in which the incident occurred.
Before contacting Appolon Bioteck technical support, please collect the following information:
- Product name,
- Batch number,
- Error message (if any)

Please keep the "runs" (screen of the fluorescence before passing the sample test) to enable the manufacturer to identify the incident encountered with the kit.

16. DISPOSAL OF THE PRODUCT AFTER USE

For disposal of this product, please refer to current national and/or international regulations governing the disposal of hazardous, biological or infectious-risk waste.
Unused kit reagents can be considered non-hazardous and disposed of in accordance with normal laboratory procedures.

17. DISCLAIMER

No part of this document may be reproduced or transmitted in any form without the written permission of Appolon Bioteck.
Appolon Bioteck reserves the right to modify its products and services at any time. Appolon Bioteck assumes no liability for errors or omissions or for any damages arising from the application or use of this information.

18. SYMBOLS AND LOGOS USED

Symbol	Meaning	Symbol	Meaning
	Manufacturer		Catalogue reference number of the product
	Lot number		Contains sufficient material for <n> tests
	Expiry Date		Do not use if package is damaged and consult instructions for use
 *(https://appolonbioteck.com)*	Consult electronic instructions for use		Storage temperature limit
	Caution and consult electronic instructions for use		Keep dry
	Keep away from sunlight		Made for Food Quality control

19. HISTORY OF CHANGES TO THE INSTRUCTION FOR USE

Version date	Change
2024-10	Document release

CUSTOMER CONTACT

For any questions concerning additional documentation (Safety Data Sheets, Certificates of Analysis, etc.), please contact Appolon Bioteck by phone or e-mail:

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- E-mail : support@appolonbioteck.com

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