

# Meat ID™ Halal

Kits for screening and detection of meat species via qPCR

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## INSTRUCTIONS FOR USE

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**FOR USE IN RESEARCH AND QUALITY CONTROL**

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## Symbols

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**Lot No.**



**Order No.**



**Expiry date**



**Storage temperature**



**Number of reactions**



**Manufacturer**

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## INDICATION

The identification of different meat types, especially in minced meat products is a serious task in food safety and from an ethical perspective, especially for Muslims. Authentication of forbidden or non-declared ingredients such as pork or substandard meat is essential to ensure confidence in the supply chain and the regulatory compliance.

Meat ID™ Halal is a kit for the fast and reliable identification of pork and donkey contaminations in meat and other foods by qPCR, and is provided as lyophilized ready to use master mix. Meat ID™ Halal is available for rapid and reliable analysis from various matrices including raw, or even highly processed and cooked meat products where the DNA may be significantly degraded. It is possible to identify pork and donkey down to a threshold level of 0.5% with a semi-quantitative result.

## TEST PRINCIPLE

The assay is based on the TaqMan® principle and relies on the 5'→3' exonuclease activity of Taq polymerase as well as the dual labeling of the probes with fluorescents and quenchers. During PCR the Taq polymerase cleaves and removes annealed probes releasing the previously quenched fluorescent signal. TaqMan® based qPCR is compatible with both FAM™ and HEX™ labeled probes.

The target sequence is a mitochondrial multi-copy gene (cytochrome b). Therefore, occurrence of even small amounts of DNA (down to 1 pg/PCR-reaction) could lead to positive results. Primer sets are specific to the animal species tested.

False-negative results caused by PCR inhibition and/or DNA extraction issues will be reliably identified by means of the Internal Control DNA. The Internal Control DNA is either added directly to the PCR master mix to function as a PCR amplification control, or is alternatively added to the original sample prior to DNA extraction. By adding the Internal Control DNA directly to the sample, both DNA extraction and qPCR amplification are validated. The internal control amplification is detected at 560 nm (HEX channel), whereas the species-specific amplification is detected at 520 nm (FAM channel).

The reaction mixture contains dUTP instead of dTTP to facilitate the degradation of amplicon carry-over by use of uracil-DNA glycosylase (UNG). Thus, the probability of false-positive result is minimized. Please note that UNG is not included in the Meat ID™ Halal kit.

The user instructions include protocols for analysis of DNA extracted from meat samples to be tested (e.g. by using ExtractNow™ Meat ID, Cat. No. 608-1050 or user provided method). The entire assay needs approximately 90 minutes, excluding DNA extraction, and the detection by PCR is considered to be superior in terms of sensitivity.

Please note that Meat ID™ Halal kit does not provide a Halal-Compliance Declaration regarding the animal slaughter methods which underlie the Halal guidelines and regulations.

## REAGENTS

The provided lyophilized Species Master Mix contains Taq polymerase, nucleotides, and primer sets for animal species to be tested (pork and donkey). Internal Control DNA, Positive Control DNA, Rehydration buffer and PCR grade water are also provided in the kit.

Each kit contains reagents for 25 or 100 reactions. The expiry date of the unopened package is marked on the package label. The kit components can be stored at +2 to +8 °C for at least 12 months. After rehydration, reagents must be stored at ≤ -18 °C.

Component	25 reactions	100 reactions	Cap color
	Cat. No. 12-02-025	Cat. No. 12-02-100	
Species Master Mix	1 Vial, freeze-dried	4 Vials, freeze-dried	red
Rehydration Buffer	1 Vial, 1.3 ml	2 Vials à 1.3 ml	blue
Positive Control DNA	1 Vial, freeze-dried	4 Vials, freeze-dried	green
Internal Control DNA	1 Vial, freeze-dried	4 Vials, freeze-dried	yellow
PCR grade water	1 vial, 2 ml	1 Vial, 2 ml	white

The LOT-specific quality control certificate (Certificate of Analysis) can be downloaded from our website ([www.minerva-biolabs.com](http://www.minerva-biolabs.com)).

## USER-SUPPLIED CONSUMABLES AND EQUIPMENT

Meat ID™ Halal kit contains necessary reagents for setting up the PCR reaction. Additional consumables and equipment are supplied by the user:

- qPCR device with filter sets for detecting the fluorescence dyes FAM™ and HEX™
- DNase-free 0.2 ml and 1.5 ml reaction tubes
- Microcentrifuge for 1.5 ml and 0.2 ml reaction tubes
- Pipettes with corresponding filter tips (10, 100, and 1000 µl)
- Required for DNA extraction:  
DNA extraction kit, e.g. ExtractNow™ Meat ID kit (Cat. No. 608-1050), or user method, Ethanol > 96 % abs., heat block
- Optional for carry-over prevention: Uracil DNA glycosylase (UNG)
- For more details on sample preparation download the application note from our website ([www.minerva-biolabs.com](http://www.minerva-biolabs.com)).

## SAMPLES

For DNA extraction we recommend our ExtractNow™ Meat ID kit (Cat. No. 608-1050). This kit was tested intensively and optimized for the extraction of DNA from meat samples. The protocols for DNA extraction are described in detail in the „Instructions for Use“ of the DNA extraction kit. Extracted DNA may be stored at +2 to +8 °C for up to 6 days. Long term storage must be at  $\leq -18$  °C. Repeated thawing and freezing of DNA extracts should be avoided.

## RECOMMENDATIONS

If further investigation is desired, we recommend our qPCR-based Meat ID™ Screen kit, a reliable screening system for rapid and simultaneous detection of the most common meat species, i.e. goat, sheep, pork, horse, beef, donkey, fish, chicken, turkey, and camel in meat and other foods (for ordering information see Appendix II-Related Products).

Food samples should be collected according to local standard methods and guidelines. Meat ID™ Halal kit can be used in research and industry for the detection of pork and donkey in meat and other foods. Do not use for clinical or diagnostic applications or for testing of patient samples.

## PRECAUTIONS

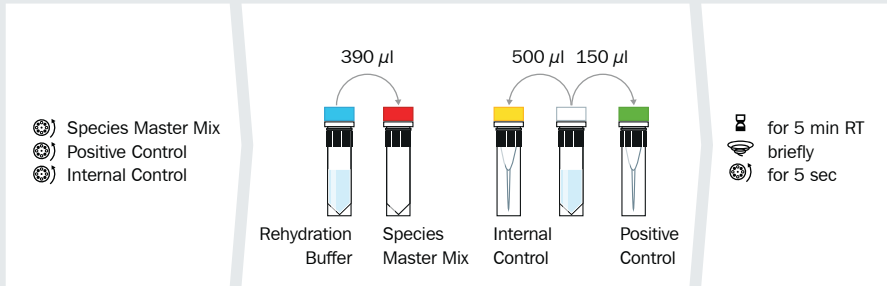
Meat ID™ Halal kit should be used by trained laboratory staff only. All samples should be handled with all due care and attention. Always wear a suitable lab coat and disposable gloves. This kit does not contain hazardous substances. Waste is disposable according to local regulations.

## IMPORTANT NOTES

- ⇒ These instructions must be understood to successfully use the Meat ID™ Halal kit. The reagents supplied should not be mixed with reagents from different batches but used as an integral unit. The reagents of the kit must not be used beyond their shelf life.
- ⇒ Follow the exact protocol. Any deviations may affect the test method and results.
- ⇒ PCR inhibition is likely to be caused by the sample matrix. Thus, we recommend our Extract-Now™ Meat ID kit for sample preparation. Any other DNA extraction kit needs to be qualified.
- ⇒ It is important to include control samples on a regular basis to monitor the reliability of your results. Positive and negative (non-template) controls are essential in case of troubleshooting and should be prepared in duplicates in each PCR. Use elution buffer for the NTC in case of extracted DNA.
- ⇒ The control samples must be processed in the same manner as the test samples. You may want to include other laboratory specific control samples such as high, median and low DNA levels.

# PROCEDURE – OVERVIEW

## 1. Reagent Preparation



## 2. Reaction Mix Preparation !

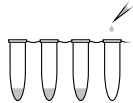
### 1 reaction

15  $\mu$ l Species Master Mix (red cap)  
 1  $\mu$ l Internal Control (yellow cap)



## 3. Loading the Test Tubes

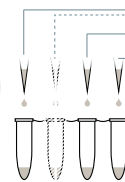
aliquot 15  $\mu$ l  
 Reaction Mix



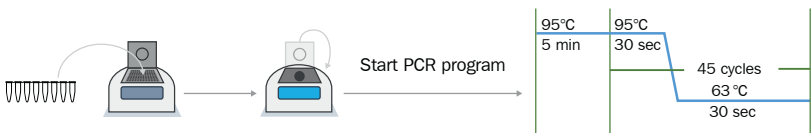
## 4. Adding Samples

+ 5  $\mu$ l DNA extract  
 + 5  $\mu$ l Positive Control (green cap)  
 + 5  $\mu$ l Elution Buffer (Negative Control)

centrifuge briefly



## 5. PCR Amplification



! If Internal Control was already added during DNA extraction, skip step 2 and proceed directly to step 3 and aliquot 15  $\mu$ l Species qPCR Mix (red cap).

- Rehydration Buffer
- Species Master Mix
- PCR grade water
- Positive Control
- Internal Control

- incubate
- vortex
- centrifuge
- +** add

storage 2-8 °C  
 after rehydration  $\leq$  -18 °C

## PROCEDURE - STEP BY STEP

All reagents and samples must be equilibrated to +2 to +8 °C prior use. After reconstitution, reagents should be stored at  $\leq -18$  °C. Repeated freezing and thawing should be avoided and reconstituted controls should be stored in aliquots.

### 1. Reagent preparation

1.	Species Master Mix	red cap	Spin down freeze-dried components at max speed for 5 sec.
	Positive Control DNA	green cap	
	Internal Control DNA	yellow cap	
2.	Species Master Mix	red cap	Add 390 $\mu$ l Rehydration Buffer (blue cap).
3.	Positive Control DNA	green cap	Add 150 $\mu$ l PCR grade water (white cap).
4.	Internal Control DNA	yellow cap	Add 500 $\mu$ l PCR grade water (white cap).
4.	Species Master Mix	red cap	Incubate at room temperature for 5 min.
	Positive Control DNA	green cap	
	Internal Control DNA	yellow cap	
5.	Species Master Mix	red cap	Vortex briefly and spin down for 5 sec.
	Positive Control DNA	green cap	
	Internal Control DNA	yellow cap	

### 2. Preparation of PCR reaction mix

The following steps (reaction mix preparation, adding samples and starting the PCR reaction) should be done in less than 45 minutes to avoid a reduction in the fluorescence signal.

Optional: for verification of the DNA extraction step, Internal Control DNA can be added to the original sample prior extraction (at least 20  $\mu$ l Internal Control DNA should be added to the sample). In this case, do not add further Internal Control DNA to the PCR mastermix, proceed directly to step 2 and aliquot 15  $\mu$ l of Species Master Mix (red cap) to each PCR reaction tube.

1. Prepare the required volume of PCR mastermix for the number of samples you wish to test. Mix the kit components at room temperature in a 1.5 ml reaction tube for all control and test reactions.				
	<b>Cap color</b>	<b>for 1 reaction</b>	<b>for 25 reactions</b>	
	Species Master Mix	red	15 $\mu$ l	375 $\mu$ l
	Internal Control DNA	yellow	1 $\mu$ l	25 $\mu$ l
2. Aliquot 15 $\mu$ l of PCR mastermix to each PCR tube, discard remaining material.				

### 3. Adding samples

Add samples according to the following pipetting sequence:

- 
1. Negative Controls: add 5  $\mu$ l H<sub>2</sub>O or elution buffer from DNA extraction kit.

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  2. Samples: add 5  $\mu$ l of extracted DNA from meat sample.

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  3. Positive Control: add 5  $\mu$ l Positive Control DNA (green cap).

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  4. Close the PCR tubes tightly and spin down briefly.

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### 4. Start PCR amplification

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1. Place PCR tubes in the qPCR device and close the lid.

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  2. Program the qPCR cycler (see appendix I for detailed cycler programs of selected qPCR cyclers. Programs for additional cyclers are available upon request).

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  3. Start the program.

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This assay was tested on the following qPCR devices:

qPCR device	Manufacturer
CFX-96™	Bio-Rad Laboratories
ABI Prism® 7500	Applied Biosystems
RotorGene® 6000	Corbett Research
Mx3005P®	Agilent Technologies



## DATA INTERPRETATION

The exact procedure for obtaining Ct-values including baseline calculation/normalization depends on the particular qPCR device and cycler control software. Please see the documentation of your device for further details. We recommend the assessment of the amplification curve progression of all samples including control samples.

A positive PCR is indicated by  $C_t < 40$ . PCR reactions with  $C_t \geq 40$  are considered negative. In addition, a successful PCR is displayed by an increasing fluorescence signal in either the FAM™ or the HEX™ channel (for the Internal Control), or both. The presence of unwanted animal species in meat samples is indicated by an increasing fluorescence signal in the FAM channel. The animal-species DNA under investigation and the Internal Control function as competitors in the PCR. Thus, the more DNA of the species under investigation is in the sample, the higher the signal in the FAM™ channel and the lower the internal control signal in the HEX™ channel. The following table will help with the interpretation of PCR results:

Detection of Animal Species FAM™ channel	Internal control HEX™ channel	Interpretation
positive	irrelevant	Animal species positive
negative	negative	PCR inhibition
negative	positive	Animal species negative

## ASSAY CHARACTERISTICS

### 1. Sensitivity

It is possible to detect the target meat-type in the environment of other meat types down to a threshold level of 0.5 % with a detection sensitivity of down to 10 DNA copies per PCR, when this kit is combined with ExtractNow™ Meat ID DNA extraction kit, which was specially optimized for the extraction of DNA from meat samples.

### 2. Specificity

No cross-reactivity with DNA of other animal species could be observed.

## APPENDIX I

The following protocols were created based on internal experience and customer reports. Minerva Biolabs does not warrant or assume responsibility for the performance of these protocols.

### Programming the LightCycler® 2.0

#### Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
<b>Temperature Targets</b>	<b>Segment 1</b>
Target Temperature [°C]	95
Incubation time [min]	5:00
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

#### Program 2: Amplification

Cycles	45	
Analysis Mode	Quantification	
<b>Temperature Targets</b>	<b>Segment 1</b>	<b>Segment 2</b>
Target Temperature [°C]	95	63
Incubation time [s]	30	30
Temperature Transition Rate [°C/s]	20.0	20.0
Secondary Target Temperature [°C]	0	0
Step Size [°C]	0.0	0.0
Step Delay [Cycles]	0	0
Acquisition Mode	None	Single

#### Program 3: Cooling

Cycles	1
Analysis Mode	None
<b>Temperature Targets</b>	<b>Segment 1</b>
Target Temperature [°C]	40
Incubation time [s]	30
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

#### Result Reading:

- Select the fluorescence channels 1 and 2
- Click on *Quantification* to generate amplification plots and Ct-values
- The threshold will be generated automatically.
- Samples showing no significant increase in the amplification plot can be considered as negative.

## Programming of ABI Prism® 7500

### Detector Settings:

Target Probe: Reporter - FAM / Quencher - none  
Internal Control Probe: Reporter - HEX / Quencher - none

The “ROX Reference” function needs to be disabled, as no ROX dye is included in the mix. Activate both detectors for each well.

Measure fluorescence during annealing.

### Program Step 1: Pre-incubation

Setting Hold  
Temperature 95 °C  
Incubation time 5:00 min

### Program Step 2: Amplification

Cycles 45  
Setting Cycle  
Denaturing 95 °C for 30 sec  
Annealing 63 °C for 30 sec & data reading

### Result Reading:

- Enter the following basic settings at the right task bar:  
Data: Delta RN vs. Cycle  
Detector: FAM and HEX  
Line Color: Well Color
- Open a new window with for the *Graph settings* by clicking the right mouse button  
Select the following setting and confirm with *ok*:  
Real Time Settings: Linear  
Y-Axis Post Run Settings: Linear and Auto Scale  
X-Axis Post Run Settings: Auto Scale  
Display Options: 2
- Initiate the calculation of the Ct-values and the graph generation by clicking on *Analyze* within the report window.
- Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no Ct-value can be considered as negative.

## Programming of RotorGene® 6000 (5-plex)

### Program Step 1: Pre-incubation

Setting	Hold
Hold Temperature	95 °C
Hold Time	5 min 0 sec

**Please check the correct settings for the filter combination:**  
**green filter (510):**           **Animal-Species**  
**yellow filter (555):**       **Internal Control**

### Program Step 2: Amplification

Setting	Cycling
Cycles	45
Denaturation	95°C for 30 sec
Annealing	63°C for 30 sec → <b>acquiring to Cycling A (green and yellow)</b>
Gain setting	automatic (auto Gain)
Slope Correct	activated
Ignore First	deactivated

### Result Reading:

- Open the menu *Analysis*
- Select *Quantitation*
- Check the required filter set (green and yellow) according to the following table and start data analysis by double click.
- The following windows will appear:

*Quantitation Analysis - Cycling A (green or yellow)*

*Quant. Results - Cycling A (green or yellow)*

*Standard Curve - Cycling A (green or yellow)*

- In window *Quantitation Analysis*, select first linear scale and then *slope correct*  
Threshold setup (not applicable if a standard curve was included in parallel and auto threshold was selected)  
- In window *CT Calculation* set the threshold value to 0-1  
- Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- The Ct-values can be taken from the window *Quant. Results*.

## Programming the Mx3005P®

- Go to the setup menu, click on „Plate Setup“, check all positions which apply
- Click on „Collect Fluorescence Data“ and check FAM and HEX
- Corresponding to the basic settings the „Reference Dye“ function should be deactivated
- Specify the type of sample (negative or positive control, sample, standard) at „well type“
- Edit the temperature profile at „Thermal Profile Design“:

Segment 1:                    95 °C for 5 min

Segment 2:

Denaturing                    95 °C for 30 sec

Annealing                    63 °C for 30 sec & data collection end

45 cycles

- at menu „Run Status“ select „Run“ and start the cycler by pushing „Start“

### Analysis of raw data:

- In the window „Analysis“ tab on „Analysis Selection / Setup“ to analyze the marked positions
- Ensure that in window „algorithm enhancement“ all options are activated:  
Amplification-based threshold  
Adaptive baseline  
Moving average
- Click on „Results“ and „Amplification Plots“ for an automatic threshold
- Read the Ct values at „Text Report“

## Programming the LightCycler® 480

### Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
<b>Temperature Targets</b>	<b>Segment 1</b>
Target Temperature [°C]	95
Incubation time [min]	5:00
Temperature Transition Rate [°C/s]	4.4
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

### Program 2: Amplification

Cycles	45	
Analysis Mode	Quantification	
<b>Temperature Targets</b>	<b>Segment 1</b>	<b>Segment 2</b>
Target Temperature [°C]	95	63
Incubation time [s]	30	30
Temperature Transition Rate [°C/s]	4.4	2.2
Secondary Target Temperature [°C]	0	0
Step Size [°C]	0.0	0.0
Step Delay [Cycles]	0	0
Acquisition Mode	None	Single

### Program 3: Cooling

Cycles	1
Analysis Mode	None
<b>Temperature Targets</b>	<b>Segment 1</b>
Target Temperature [°C]	40
Incubation time [s]	30
Temperature Transition Rate [°C/s]	2.2
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Before starting the LC480, make sure that the filter setting is correct:

LightCycler® 480	Animal-Species	Internal Control
Instrument I	533 nm	568 nm
Instrument II	510 nm	580 nm

# Programming the CFX 96 Touch™, CFX96 Touch Deep Well™, CFX Connect™, and CFX384 Touch™ (Bio-Rad)

## Performing Runs

### *Run Setup - Protocol Tab*

- Click **Create New** to open the Protocol Editor to create a new protocol.
- Select any step in either the graphical or text display. The selected step becomes highlighted in blue. Click the temperature or incubation time to directly edit the value.

	Step 1	Step 2	Step 3	Step 4
Temperature	95.0 °C	95.0 °C	63.0 °C	GO TO STEP 2
Incubation time	05:00 min	00:30 sec	00:30 sec	× 45

### *Run Setup - Plate Tab*

- Click **Create New** to open the Plate Editor to create a new plate.
- Use the **Scan Mode** dropdown menu in the Plate Editor toolbar to designate the data acquisition mode to be used during the run. Important!!! Select the **All Channels** mode.
- Click the **Select Fluorophores** button to indicate the fluorophores that will be used in the run.
- Select the wells to be loaded within the plate diagram.

### *Run Setup – Start Run Tab*

- View the selected Protocol file, Plate file, and data acquisition Scan Mode setting in the **Run Information** pane.
- Select one or more blocks and edit run parameters if necessary in Start Run on Selected Block(s) pane.
- Click the Start Run button to begin the run.

## Data Analysis

### *Quantification Tab*

The amplification chart data in this tab display the relative fluorescence (RFU) collected from each well at every cycle of the run.

- Choose the fluorophore data you want to display by clicking the fluorophore checkboxes located under the amplification chart.

- The Software uses two modes for quantification cycle determination. Select **Settings** from the menu bar and select **Baseline Subtracted Curve Fit** as baseline setting and **Single Threshold Mode** as Cq Determination Mode.
- In the **Single Threshold Mode**, click and drag the threshold line to manually position the line. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no C<sub>t</sub>-value can be considered as negative.

## APPENDIX II

### *Limited Product Warranty*

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from of the use, the results of use, or the inability to use this product.

### *Trademarks*

LightCycler is a registered trademark of a member of the Roche Group. ABI Prism is a registered trademark of Applied Biosystems Corporation or its subsidiaries in the US and certain other countries. CFX96 Touch, CFX96 Touch Deep Well, CFX Connect, and CFX384 Touch are trademarks of Bio Rad Laboratories. TaqMan is a registered trademark of Roche Molecular Systems, Inc. FAM, and HEX are trademarks of Applied Biosystems LLC. Venor is a registered trademark and Food Control, Meat ID, Vegan Control, ExtractNow, PCR Clean, Lab Clean, WaterShield, ConviFlex, SwabUp, and Cyclor Check are trademarks of Minerva Biolabs GmbH, Germany.



## Related Products

### qPCR Kits for Food Control

11-02-XX-025	Food Control™ qPCR	25 reactions
11-03-XX-025	FoodControl™ LFA	25 tests
11-04-XX-025	FoodControl™ LFA+	25 tests

### qPCR Kits for Meat Identification

12-02-025/-100	Meat ID™ Screen	5/20/40 reactions
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### qPCR Kits for Vegan Control

12-05-025/-100	VeganControl™	25/100 reactions
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### DNA Extraction kits

56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
56-2096	Venor®GeM Sample Preparation Kit - IP C16	96 extractions
601-1010/1050/1200	ExtractNow™ DNA Mini Kit	10/50/200 extractions
602-1010/1050/1200	ExtractNow™ Blood DNA Mini kit	10/50/200 extractions
603-1010/1050/1200	ExtractNow™ RNA Mini kit	10/50/200 extractions
604-1010/1050/1200	ExtractNow™ Cleanup kit	10/50/200 extractions
605-1010/1050/1200	ExtractNow™ Plasmid Mini kit	10/50/200 extractions
606-1010/1050/1200	ExtractNow™ Virus DNA/RNA kit	10/50/200 extractions
607-1010/1050	ExtractNow™ Vegan Control	10/50 extractions
608-1010/1050	ExtractNow™ Meat ID	10/50 extractions
609-1010/1050	ExtractNow™ Food Control	

### PCR Clean™ (formerly DNA Remover™)

15-2025/2200	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4x 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 x 120 wipes in a bag
15-2203	Wipes, single wrapped	30 Sachets

### Lab Clean™

15-4100	Molecular Microbiology Lab Cleaner	1 liter
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### WaterShield™

15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200x concentrate	30 x 5 ml/3 x 50 ml/500 ml
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### ConviFlex™ DNAmix

191-025/100/250	PCR Mix with Taq polymerase for conventional and qPCR	25/100/250 reactions
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### SwabUp™ Lab Monitoring Kits

181-0010/0050	Sample collection and DNA extraction	10/50 samples
182-0010/0050	Sample collection, DNA extraction and PCR system	10/50 samples

### PCR Cycler Validation

57-2102	PCR Cycler Check™ Advance	6 strips, 8 vials each
57-2103	PCR Cycler Check™ OneStep	100 reactions
57-2202	qPCR Cycler Check™	100 reactions

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