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RapidFinder™ Vegan ID Kit

USER GUIDE

Real-time PCR detection of animal DNA in food and feed samples

for use with:
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR Instrument
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument

Catalog Number A24412
Publication Number MAN0018302
Revision D

imegenagro

For testing of Food and Environmental samples only.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: none (English)

Revision	Date	Description
D	26 September 2024	The recommendation to include a 10% overage has been removed from the protocol for setting up the components for PCR reactions.
C.0	28 November 2023	• The manufacturer address was updated. • The trademark statement was updated. • The Kit sensitivity and specificity table was updated. • The storage temperature for the General Master Mix was updated. • An exclusion for terrestrial insects was added. • A note was added for real-time PCR instrument dye calibration. • References to RapidFinder™ Analysis Software and RapidFinder™ Express Software were removed. • The KingFisher™ Flex Purification System with 96 Deep-Well Head was added.
B.0	15 July 2021	• ISO certification was added. • Invertebrate species exclusion was added. • QuantStudio™ 5 Real-Time PCR Instrument with Thermo Scientific™ RapidFinder™ Analysis Software v1.2 or later was added.
A.0	15 March 2021	New document converted from Imegen document for the RapidFinder™ Vegan ID Kit.

The information in this guide is subject to change without notice.

Contents

1 Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

Identification of meat species present in food samples is an essential step for verification of origin and traceability of raw materials, as well as for quality control of handling and cleaning processes in production lines. The Thermo Scientific™ RapidFinder™ Vegan ID Kit enables real-time PCR detection of ≥0.01% of animal DNA in food and feed samples. Invertebrate animal species such as seafood (crustaceans and molluscs) and terrestrial insects such as worms, crickets, and grasshoppers are not detected by the assay.

The kit includes:

- All reagents necessary for the real-time PCR reaction—specific FAM™-labeled probe and primers for animal mitochondrial DNA, DNA polymerase enzyme, and other buffer components.
- An internal positive control (IPC)—VIC™-labeled probe, primers, and template, to monitor for PCR inhibition (included in the reagents).
- A Positive Control, to confirm animal DNA detection.

Unknown samples and control samples are provided by the investigator.

Note: The qPCR instrument must be calibrated with the following dyes before use: FAM™ and VIC™.

Kit contents and storage

Table 1 RapidFinder™ Vegan ID Kit (Cat. No. A24412)

Component	Amount (48 reactions)	Storage[1]
Vegan Master Mix (black pad)	360 µL	–20°C
General Master Mix (white pad)	600 µL	–20°C upon receipt. 2–8°C after initial use. Store protected from light.
Positive Control (orange cap)	60 µL	–20°C

[1] See the expiration date on the box.

Materials required but not provided

Unless otherwise indicated, all materials are available through the Thermo Fisher Microbiology ordering process or **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
Real-time PCR instrument, one of the following:	
Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System	Contact your local microbiology sales representative.
Applied Biosystems™ 7500 Fast Real-Time PCR System	
Recommended equipment for automated DNA isolation	
KingFisher™ Flex Purification System with 96 Deep-Well Head	A32681
Other equipment	
Adjustable micropipettors (10 µL, 20 µL, 200 µL)	Available through the Thermo Fisher Microbiology ordering process. See **thermofisher.com/plastics** for more information.
Benchtop microcentrifuge with adaptors for PCR plates and/or tubes	
Laboratory mixer (Vortex mixer or equivalent)	
Optical reaction plates and covers, *or* optical PCR tubes and caps	
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Optical Adhesive Film, 100 covers	4311971
MicroAmp™ Fast 8-Tube Strip, 0.1 mL (See below for caps.)	4358293
MicroAmp™ Optical 8-Cap Strips	4323032
Other plastics and consumables	
Aerosol-resistant pipette tips	Available through the Thermo Fisher Microbiology ordering process. See **thermofisher.com/plastics** for more information.
1.5-mL nuclease-free microcentrifuge tubes	
Powder-free disposable gloves	
Reagents	
Nuclease-free water (not DEPC-Treated)	AM9938
Recommended kits for DNA isolation, one of the following:	

(continued)

Item	Source
GMO Extraction Kit	4466336
For high-throughput isolation: Lysis Buffer 1 + RNase GMO Extraction Kit PrepSEQ™ Nucleic Acid Extraction Kit	A24401 4428176, 4480466

2 Methods

Input DNA requirements

- Prepare the DNA sample with a method that allows processing of 10–20 g of food sample.
  - For low-throughput, manual processing, use the GMO Extraction Kit.
  - For automated processing, it is recommended to use the Lysis Buffer 1 + RNase GMO Extraction Kit and the PrepSEQ™ Nucleic Acid Extraction Kit with the KingFisher™ Flex Purification System with 96 Deep-Well Head.
- Prepare at least one mock-purified sample as a negative extraction control, processed with the same DNA isolation method that is used for test samples.
- Dilute the final DNA sample to 10 ng/µL for the PCR.

Determine the number of reactions and thaw the reagents

1. Plan to include the following reactions:
   - A single reaction for each test sample.
   - Control reactions.
     - Positive Control (included in the kit).
     - Negative extraction control (mock-purified samples).
     - No-template control reactions; use nuclease-free water in place of sample DNA.
2. Thaw all reagents, vortex to mix thoroughly, then place on ice.

Set up the PCR reactions

1. Combine the following components for the number of reactions required.

Component	Volume per reaction
Vegan Master Mix (black pad)	7.5 µL
General Master Mix (white pad)	12.5 µL

2. Mix thoroughly by vortexing, then distribute 20 µL to each reaction well or tube.
3. Add 5 µL of DNA sample (10 ng/µL), mock-purified sample (negative extraction control), nuclease-free water (no-template control), or Positive Control to the appropriate wells.
4. Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom.

Set up and run the real-time PCR instrument

See the appropriate instrument user guide for detailed instructions to set up and run the real-time PCR instrument.

1. Set up the real-time PCR instrument using the following settings:
   - Reaction volume: 25 µL
   - Passive reference dye: ROX™ dye included
   - TaqMan™ probe reporter dyes and quenchers:

Target	Reporter	Quencher
Animal DNA	FAM™ dye	NFQ-MGB
IPC	VIC™ dye	NFQ-MGB

   - Thermal cycler settings:

Setting	Stage 1 Enzyme activation	Stage 2 PCR	
Number of cycles	1 (Hold)	36	
		Denature	Anneal/extend[1]
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	1 minute

[1] Fluorescence is acquired during the annealing/extension stage.

2. Load the reactions, run the thermal cycler program and collect real-time amplification data.

Analyze results

The general process for analyzing results is described in this section. The details of data analysis depend on the real-time PCR instrument that you use; see the appropriate user guide for instructions on how to analyze your data.

1. View the amplification plots for all reactions to make sure that they appear normal.
2. Use the **Auto** instrument setting to set the baseline.
3. Set the FAM™ and VIC™ threshold to 0.1.

4. Check that the results obtained in all control wells are as expected. For unexpected control results, see Appendix A, "Troubleshooting".

Reaction type	FAM™ channel (Animal DNA)	VIC™ channel (IPC)[1]
Positive Control	+	+
Negative extraction control	—	+
No-template control	—	+

[1] In all reactions, the C_t of the IPC should be close to the C_t of the Positive Control.

5. Establish the positive cut-off value for the test samples and assign results:

$C_{t\ (cut\text{-}off)} = C_{t\ (Positive\ Control)} + 3.32$

Sample C_t value	Sample result
$C_t > C_{t\ (cut\text{-}off)}$	Negative
$C_t \leq C_{t\ (cut\text{-}off)}$	Positive[1]

[1] For fresh or minimally processed meat samples, the cut-off value corresponds to approximately 0.01% animal DNA, when the DNA sample concentration is 10 ng/µL.

Interpretation of results

Interpret unknown sample results according to the following table.

FAM™ channel (Animal DNA)	VIC™ channel (IPC)	Interpretation
—	+	Animal DNA not detected.
+	+	Animal DNA detected.
—	—	Invalid result. See Appendix A, "Troubleshooting".
+	—	This result is expected in reactions that have a strong FAM™ signal. See Appendix A, "Troubleshooting".

A

Troubleshooting

Observation	Possible cause	Recommended action
In the Positive Control wells, no target-specific and no IPC signals are detected.	PCR amplification failed.	Check that the thermal cycler settings and amplification program are correct.
In the negative extraction control wells, target-specific and IPC signals are detected.	Contamination occurred during the DNA extraction procedure.	Contamination may be due to errors in sample handling, reagent contamination, or environmental contamination. • Check that the DNA extraction protocol was performed correctly. • Take care to avoid contamination during sample homogenization: decontaminate grinding equipment or homogenizer with 10% bleach or DNA*Zap*™ Solutions (Cat. No. AM9890). • Decontaminate benchtop surfaces and other equipment where the DNA extraction process is performed with 10% bleach or DNA*Zap*™ Solutions. • If necessary, use fresh reagents and repeat the DNA extraction.
In the no-template control wells, target-specific and IPC signals are detected.	Contamination occurred during PCR.	Contamination may be due to errors in sample handling, reagent contamination, or environmental contamination. • Decontaminate benchtop surfaces and other equipment where PCR is performed with 10% bleach or DNA*Zap*™ Solutions (Cat. No. AM9890). • Use fresh reagents and repeat the PCR. • Set up the Positive Control PCR reactions last to avoid cross-contamination.
In unknown wells, no IPC signal is detected, but target-specific signal is detected.	A high copy number of target DNA existed in the samples, resulting in preferential amplification of the target-specific DNA.	No action is required. The result is considered positive.
In unknown wells, no IPC or target-specific signal is detected.	Excess sample DNA was used in PCR; the recommended maximum is 250 ng.	Repeat the PCR with the correct amount of DNA. If DNA quantification is not possible, dilute the DNA sample.
	PCR inhibitors were present in the sample DNA.	Repeat the DNA extraction. If the problem persists, contact Technical Support.

B

Supplemental information

Kit sensitivity and specificity

The detection limit was calculated with standard samples consisting of mixtures of raw animal meat and other species. The RapidFinder™ Vegan ID Kit can detect blends with as little as 0.01% (w/w) of animal meat. The limit of detection in processed samples varies depending on the composition and food processing.

The kit specificity was tested by comparison of the probe and primer sequences with the NCBI database, and it was also experimentally tested on a collection of reference DNAs with the following results:

Table 2 Species used during the specificity assay for the RapidFinder™ Vegan ID Kit

Species	Result
Horse (*Equus caballus*)	Detected
Mule (*Equus asinus* × *Equus caballus*)	Detected
Donkey (*Equus asinus*)	Detected
Beef (*Bos taurus*)	Detected
Pork (*Sus scrofa domestica*)	Detected
Water buffalo (*Bubalus bubalis*)	Detected
Fallow deer (*Dama dama*)	Detected
Chicken (*Gallus gallus*)	Detected
Turkey (*Meleagris gallopavo*)	Detected
Goat (*Capra aegagrus hircus*)	Detected
Duck (genus *Anas*)	Detected
Ostrich (*Struthio camelus*)	Detected
Goose (*Anser anser*)	Detected
Sheep (*Ovis aries*)	Detected
Human (*Homo sapiens*)	Detected
Rabbit (*Oryctolagus cuniculus*)	Detected
Dog (*Canis familiaris*)	Detected

Table 2 Species used during the specificity assay for the RapidFinder Vegan ID Kit *(continued)*

Species	Result
Longfin tuna (*Thunnus alalunga*)	Detected
European hake (*Merluccius merluccius)*	Detected
European pilchard (*Sardina pilchardus*)	Detected
Atlantic salmon (*Salmo salar*)	Detected
Haddock (*Melanogrammus aeglefinus*)	Detected
Pollack (*Pollachius pollachius*)	Detected
Atlantic cod (*Gadus morhua*)	Detected
Mealworms (*Tenebrio molitor*)	Not Detected
Buffalo worms (*Alphitobius diaperinus*)	Not Detected
Cricket (*Acheta domesticus*)	Not Detected
Migratory locust (*Locusta migratoria*)	Not Detected
Mussel (*Mytilus galloprovincialis*)	Not Detected
Squid (*Loligo vulgaris*)	Not Detected
Prawn (*Aristaeomorpha foliacea*)	Not Detected
Wheat (*Triticum aestivum*)	Not Detected
Soya (*Glycine max*)	Not Detected
Maize (*Zea mays)*	Not Detected
Tomato (*Solanum lycopersicum*)	Not Detected
Rice (*Oryza sativa*)	Not Detected
E. coli (*Escherichia coli*)	Not Detected

UNE-EN ISO 9001 certification

Health in Code S.L. is certified against the standard UNE-EN ISO 9001:2015 "Quality management systems" for the design, development, manufacture, and commercialization of kits for genetic analysis.

UNE-EN ISO 14001 certification

Health in Code S.L. is certified against the standard UNE-EN ISO 14001:2015 “Environmental Management Systems” for the design, development, manufacture, and commercialization of kits for genetic analysis.

C Good laboratory practices for PCR

Note: Spin tubes/plates before performing PCR. Spinning of PCR tubes is most easily accomplished by using a centrifuge designed for PCR tubes or plates. Follow manufacturer instructions for loading tubes/plates.

To avoid amplicon contamination of samples, follow these guidelines when preparing or handling samples for PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Do not open reaction tubes after PCR.
- Do not autoclave reaction tubes after PCR.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA*Zap*™ Solutions (Cat. No. AM9890) according to the Thermo Fisher Scientific PCR Decontamination Protocol. After cleaning with bleach we recommend a rinse with distilled water or an ethanol solution because bleach will rust stainless steel. Note that minor discoloration of metal parts may occur.

For additional information, refer to EN ISO 22174:2005 or **www.thermofisher.com/us/en/home/life-science/pcr/real-time-learning-center/real-time-pcr-basics.html**.

Plate layout suggestions

- Separate different targets by a row if enough space is available.
- Put at least one well between unknown samples and controls if possible.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- Put positive controls in one of the outer rows or columns if possible.
- Consider that caps for PCR tubes come in strips of 8 or 12.

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

Documentation and support

Food safety support

Website: **https://www.thermofisher.com/us/en/home/industrial/food-beverage/food-microbiology-testing.html**

Health in Code website for Certificates of Analysis and other product documentation: **https://portal.imegen.es/en/certificate-of-analysis/**

Support email:

- Europe, Middle East, Africa: **microbiology.techsupport.uk@thermofisher.com**
- North America: **microbiology@thermofisher.com**

Phone: Visit **thermofisher.com/support**, select the link for phone support, then select the appropriate country from the dropdown list.

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Related documentation

Document	Publication number
KingFisher™ Flex Purification System User Guide	MAN0019870
QuantStudio™ 3 and 5 Real-Time PCR Systems Installation, Use, and Maintenance Guide	MAN0010407
Applied Biosystems™ 7300/7500/7500 Fast Real-Time PCR System Installation and Maintenance Guide	4378657
Applied Biosystems™ 7500/7500 Fast Real-Time PCR System: Maintenance Guide	4387777

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.