Thermo SCIENTIFIC

TaqMan™ GMO Screening Kit

USER GUIDE

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

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imegenagro

For testing of Food and Environmental samples only.

ThermoFisher SCIENTIFIC

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About this guide

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

Revision history

Revision	Date	Description
A.0	October 2015	New document. Reformatted Rev. 02-09-2014 from Imegen.

Product information

Product description

The Thermo Scientific™ TaqMan™ GMO Screening Kit enables real-time PCR detection of genetically modified organisms (GMOs). It detects the most commonly used regulatory elements in genetically modified plants:

- The 35S promoter (P35S) from cauliflower mosaic virus (CaMV).
- The NOS terminator (TNOS) from *Agrobacterium tumefaciens*.
- The 34S promoter (P34S) from figwort mosaic virus (FMV).

These regulatory elements are used in the majority of GMOs approved by the EU and are described in GMO databases throughout the world.

P35S and TNOS are traditionally analyzed to screen for transgenic material in foods. P34S is analyzed to identify the presence of:

- MON89788 soy
- H7-1 sugar beet
- GT73 rape
- Other genetically modified crops that use this more recently adopted promoter element

The regulatory elements present in GMOs are found naturally in the viruses and bacteria from which they were originally obtained. To reduce the chance of false positive results from these naturally occurring microbes, the TaqMan™ GMO Screening Kit enables concurrent detection of genomic regions exclusive to these three microbes. This enables users to verify that the positive results are from the presence of genetically modified material and not due to the natural presence of one of the original source microbes. Results can therefore be interpreted as the presence of genetically modified material or potentially due to the natural presence of material from these microbes.

The TaqMan™ GMO Screening Kit includes primers, TaqMan™ probes, and required reagents for detection of P35S, TNOS, and P34S sequences, as well as a ubiquitous plant target. The kit includes a Positive Control containing the templates for the P35S, TNOS, and P34S regulatory elements, the CaMV, *A. tumefaciens*, and FMV genomic regions, and plant target. An internal positive control (IPC) is also included.

The PCR detection limit of the TaqMan™ GMO Screening Kit is five DNA copies per reaction for each of the regions analyzed by the kit. The kit enables the detection of 0.1% or less of GMO plant species in a background of non-GMO material, as demonstrated by the use of certified GMO reference standards.

Principle of the screening procedure

For each sample, four PCR reactions are performed. Each reaction is a multiplex PCR using the FAM™ and VIC™ channels.

PCR Master Mix	FAM™ target	VIC™ target
P35S Master Mix	P35S	CaMV genomic
TNOS Master Mix	TNOS	*A. tumefaciens* genomic
P34S Master Mix	P34S	FMV genomic
Plant Master Mix (control reaction)	Plant	IPC (internal positive control)

In the GMO target reactions:

- A positive result for the regulatory region target (FAM™) but not the genomic region (VIC™) indicates transgenic material.
- A positive result in both FAM™ and VIC™ indicates that transgenic material cannot be confirmed.

See "Analyze results" on page 12 for more information.

Kit contents and storage

Table 1 TaqMan™ GMO Screening Kit (Cat. no. 4466334)

Component	Amount (48 reactions)	Storage[1]
P35S Master Mix (red disc)	396 µL	–20°C
TNOS Master Mix (blue disc)	396 µL	–20°C
P34S-FMV Master Mix (yellow disc)	396 µL	–20°C
Plant Master Mix (green disc)	396 µL	–20°C
General Master Mix (white disc)	3 × 880 µL	4°C
Positive Control (orange cap)	250 µL	–20°C

[1] Refer to 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 through **thermofisher.com**. MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Item	Source
Instrument and equipment	
Applied Biosystems™ real-time PCR thermal cycler and required accessories: • StepOne™ Real-Time PCR System • StepOnePlus™ Real-Time PCR System • 7500 Fast Real-Time PCR System • 7500 Real-Time PCR System	Contact your local sales office.
Adjustable micropipettors (10 µL, 20 µL, 200 µL)	MLS
Benchtop microcentrifuge with adaptors for PCR plates and/or tubes	MLS
Laboratory mixer (Vortex mixer or equivalent)	MLS
Optical reaction plates and covers, *or* optical PCR tubes and caps, as appropriate for your instrument	
For use with the 7500 Real-Time PCR System:	
MicroAmp™ Optical Reaction Plate with barcode	Cat. no. 4306737
MicroAmp™ Optical Adhesive Film	Cat. no. 4311971
MicroAmp™ Fast 8-Tube Strip, 0.1 mL (See below for caps.)	Cat. no. 4358293
For use with the StepOne™ Real-Time PCR System:	
MicroAmp™ Fast Optical 48-Well Reaction Plate	Cat. no. 4375816
MicroAmp™ 48-Well Optical Adhesive Film	Cat. no. 4375323
MicroAmp™ Optical 8-Tube Strip (See below for caps.)	Cat. no. 4316567
For use with the StepOnePlus™ Real-Time PCR System or 7500 Fast Real-Time PCR System:	
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	Cat. no. 4346907
MicroAmp™ Optical Adhesive Film	Cat. no. 4311971
MicroAmp™ Fast 8-Tube Strip, 0.1 mL (See below for caps.)	Cat. no. 4358293
For use with all specified real-time PCR systems:	

Item	Source
MicroAmp™ Optical 8-Cap Strips	Cat. no. 4323032
Other plastics and consumables	
Aerosol-resistant pipette tips	MLS
1.5-mL nuclease-free microcentrifuge tubes	MLS
Powder-free disposable gloves	MLS
Reagents	
Nuclease-Free Water (not DEPC-treated)	Cat. no. AM9938
Recommended kits for DNA isolation	
GMO Extraction Kit	Cat. no. 4466336
For high-throughput isolation: Lysis Buffer 1 + RNase for Food ID PrepSEQ™ Nucleic Acid Extraction Kit	Cat. nos. A24401, 4428176, 4480466

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 high-throughput, automated processing, use Lysis Buffer 1 + RNase for Food ID and the PrepSEQ™ Nucleic Acid Extraction Kit with the MagMAX™ Express-96 or BeadRetriever™ System.
- 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 10–25 ng/µL for the PCR.

Determine the number of reactions and thaw the reagents

1. Plan to include the following reactions:
   - Four reactions for each test sample.
   - Four reactions for each control.
     - 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, and place on ice.

Set up the PCR reactions

1. Prepare a reaction mix for each PCR series, for the number of samples and control reactions as required, plus 10% overage.

Component	PCR target			
	P35S	TNOS	P34S	Plant
P35S Master Mix (red disc)	7.5 µL	—	—	—
TNOS Master Mix (blue disc)	—	7.5 µL	—	—
P34S Master Mix (yellow disc)	—	—	7.5 µL	—
Plant Master Mix (green disc)	—	—	—	7.5 µL
General Master Mix (white disc)	12.5 µL	12.5 µL	12.5 µL	12.5 µL

2. Mix thoroughly by vortexing, and distribute 20 µL to each reaction well or tube. See Figure 1 for an example plate layout.

3. Add 5 µL of DNA sample (10-25 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.

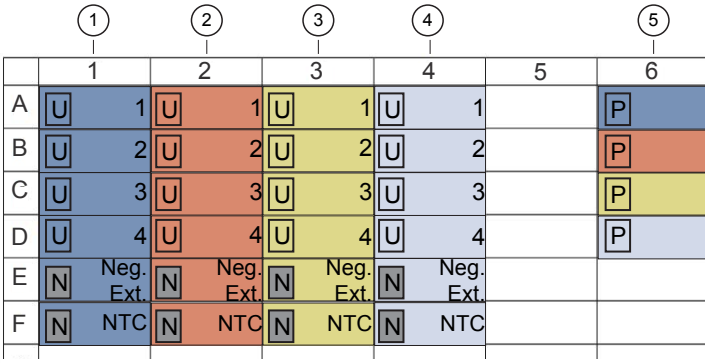

Figure 1 Example plate layout

In this example, each PCR series includes four unknown samples (U), negative extraction controls (N), no-template controls (N), and a Positive Control (P).

① P35S/CaMV
② TNOS/*A. tumefaciens*
③ P34S/FMV
④ Plant/IPC
⑤ Positive Control for each series

Set up and run the real-time PCR instrument

1. Following the manufacturer's instructions, set up the run using the following parameters:
   - Reaction volume: 25 µL
   - ROX™ passive reference dye: included
   - TaqMan™ probe reporter dyes and quenchers:

Target	Reporter	Quencher
P35S, TNOS, P34S, or plant DNA	FAM™ dye	NFQ-MGB
CaMV, *A. tumefaciens*, FMV, or IPC	VIC™ dye	NFQ-MGB

- Thermal cycler settings:

Setting	Stage 1 Enzyme activation	Stage 2 PCR	
Number of cycles	1 (Hold)	50	
		Denature	Anneal/extend
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	1 minute

Note: Settings have been optimized for the following Applied Biosystems™ real-time PCR thermal cyclers: StepOne™ Real-Time PCR System, StepOnePlus™ Real-Time PCR System, 7500 Fast Real-Time PCR System, and 7500 Real-Time PCR System.

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

Analyze results

1. Confirm that results for the Positive Control and negative controls are as expected.

Plant Master Mix		P35 Master Mix		TNOS Master Mix		P34S-FMV Master Mix		Interpretation
Plant	**IPC**	**P35S**	**CaMV**	**TNOS**	***A. tumefaciens***	**P34S**	**FMV**	
Positive Control								
+	+	+	+	+	+	+	+	Expected result
–	–	–	–	–	–	–	–	Amplification error[1]
Negative extraction control								
–	+	–	–	–	–	–	–	Expected result
+	+	–	–	–	–	–	–	Contamination with plant material[1]
+	+	+ or –[2]	–	+ or –[2]	–	+ or –[2]	–	Contamination with transgenic material[1]
No-template control (NTC)								
–	+	–	–	–	–	–	–	Expected result
+	+	–	–	–	–	–	–	Contamination with plant DNA[1]
+	+	+ or –[2]	–	+ or –[2]	–	+ or –[2]	–	Contamination with transgenic material DNA[1]
+	+	+	+	+	+	+	+	Contamination, possibly with positive control[1]

[1] See Appendix A, "Troubleshooting".

[2] One or more of the GMO targets is positive; other GMO targets can be negative.

2. Interpret unknown sample results according to the following table.

Plant Master Mix		P35S Master Mix		TNOS Master Mix		P34S-FMV Master Mix		Interpretation
Plant	**IPC**	**P35S**	**CaMV**	**TNOS**	***A. tumefaciens***	**P34S**	**FMV**	
+	+	–	–	–	–	–	–	No transgenic material containing P34S, TNOS, or P34S detected
+	+	+	–	–	–	–	–	Transgenic material containing P35S detected
+	+	–	–	+	–	–	–	Transgenic material containing TNOS detected
+	+	–	–	–	–	+	–	Transgenic material containing P34S detected
+	+	+	+	–	–	–	–	CaMV present in sample
+	+	–	–	+	+	–	–	*A. tumefaciens* present in sample
+	+	–	–	–	–	+	+	FMV present in sample
–	–	–	–	–	–	–	–	PCR inhibitors present in sample[1]
–	+	–	–	–	–	–	–	No plant DNA in sample
+	–	–	–	–	–	–	–	Sample contains large amount of plant DNA[1]

[1] 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 failure.	Check that the thermal cycler settings and amplification program are correct.
In negative extraction control wells, plant or target-specific signals are detected.	Contamination with plant or transgenic material 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, plant or target-specific signals are detected.	Contamination of the 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. See Appendix B, "Good laboratory practices for PCR and RT-PCR".
In unknown wells, plant signal is detected but no IPC signal is detected.	A large amount of plant DNA is detected, resulting in preferential amplification of the plant DNA.	No action is required.
In unknown wells, no plant, IPC, or target-specific signals are detected.	Excess sample DNA in the PCR; the recommended maximum is 250 ng.	Repeat the PCR with the correct amount of DNA.
	PCR inhibitors in the sample DNA.	Repeat the DNA extraction. If the problem persists, contact Technical Support.

B Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-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.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA*Zap*™ Solutions (Cat. no. AM9890).

For additional information, refer to ISO 22174 (2005).

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.

C

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, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

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, and 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 adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's 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 necessary) 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.

Biological hazard safety

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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may 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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Food Safety support

Website: **thermoscientific.com/foodmicro** or **thermofisher.com/foodsafety**

Imegen website for Certificates of Analysis and other product documentation: **imegen.es/cms_kits_for_analysis_food.php**

Support email: **foodsafety@lifetech.com**

Phone number in North America: 1-800-500-6855

Phone number outside of North America: Visit **thermofisher.com/support**, select the link for phone support, and select the appropriate country from the dropdown menu.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - 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.

Limited product warranty

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