Thermo SCIENTIFIC

TaqMan™ GMO Maize Quantification Kit

USER GUIDE

Real-time PCR quantification of GMO maize 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. 08-03-2011 from Imegen.

Product information

Product description

The 35S promoter (P35S) from the cauliflower mosaic virus (CaMV) is used in a large number of GMO crops, particularly maize. The Thermo Scientific™ TaqMan™ GMO Maize Quantification Kit enables relative quantification of as little as 0.01% of P35S sequence with respect to total maize in a sample.

The relative limit of quantification varies depending on the sample. The limit of quantification is 20 copies of DNA, and the detection limit of the PCR technique is 3 copies each of P35S and maize DNA. If the sample contains maize genetic modification (GM) events with several copies of P35S or another transgenic vegetable species containing P35S, the amount of transgenic material can be overestimated.

The TaqMan™ GMO Maize Quantification Kit includes:

- Primers and TaqMan™ probes for real-time PCR detection of:
  - The P35S promoter
  - The endogenous maize gene, MSS
- P35S Standard, a plasmid DNA quantitation standard containing both P35S and MSS targets
- Enzyme and other buffer components necessary for real-time PCR

Principle of the relative quantification procedure

1. Two real-time PCR series are performed:
   - One detects the P35S promoter.
   - One detects the endogenous maize gene, MSS.
   - Each PCR series includes a dilution series of the P35S Standard in addition to the unknown samples and controls.
2. For each sample, P35S and MSS targets are quantified relative to the P35S Standard.
3. The percentage of P35S target with respect to MSS target is then calculated for that sample.

Kit contents and storage

Table 1 TaqMan™ GMO Maize Quantification Kit (Cat. no. 4481972)

Component	Amount (50 reactions)	Storage[1]
P35S Master Mix (blue disc)	375 µL	–20°C
Maize Master Mix (red disc)	375 µL	–20°C
General Master Mix (white disc)	2 × 625 µL	4°C
P35S Standard (blue cap)	4 × 50 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:	

Item	Source
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:	
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. For each PCR series, plan to include the following reactions (see Figure 2):
   - Reactions for each unknown sample
   - Freshly prepared dilution series of the P35S Standard (5 dilutions)
   - Negative extraction control (mock-purified samples)
   - No-template control reactions (NTC); use Nuclease-Free Water in place of sample DNA
2. Thaw all reagents, vortex to mix thoroughly, and place on ice.

Prepare a dilution series of the P35S Standard

Prepare 1:10 serial dilutions of the P35S Standard in Nuclease-Free Water, as described in the following figure.

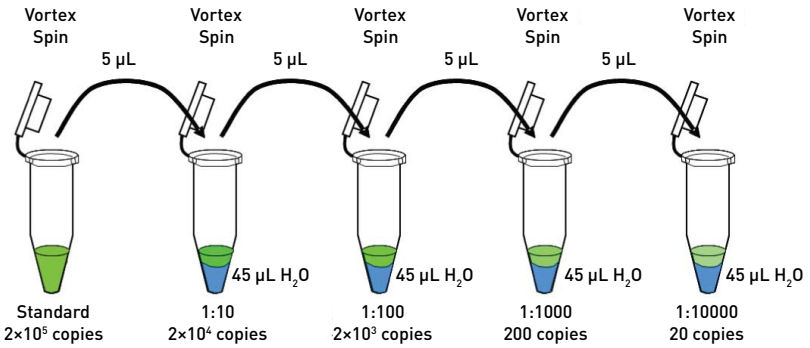

Figure 1 Serial dilution of the P35S Standard

The 1:10 serial dilutions are used to prepare standard curves for P35S and MSS. The number of copies is per 5 µL, or per PCR reaction.

Set up the PCR reactions

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

Component	PCR target	
	P35S	MSS
P35S Master Mix (blue disc)	7.5 µL	—
Maize Master Mix (red disc)	—	7.5 µL
General Master Mix (white disc)	12.5 µL	12.5 µL

2. Mix thoroughly by vortexing, and distribute 20 µL to the appropriate reaction wells or tubes. See Figure 2 for an example plate layout.

3. Add 5 µL of each sample DNA (10–25 ng/µL), mock-purified sample (negative extraction control), or Nuclease-Free Water (no-template control) to the appropriate wells for both P35S and MSS PCRs.

4. Add 5 µL of each P35S Standard dilution to the appropriate wells for both P35S and MSS PCRs.

5. Seal each plate or tube, mix, then centrifuge briefly to bring the contents to the bottom.

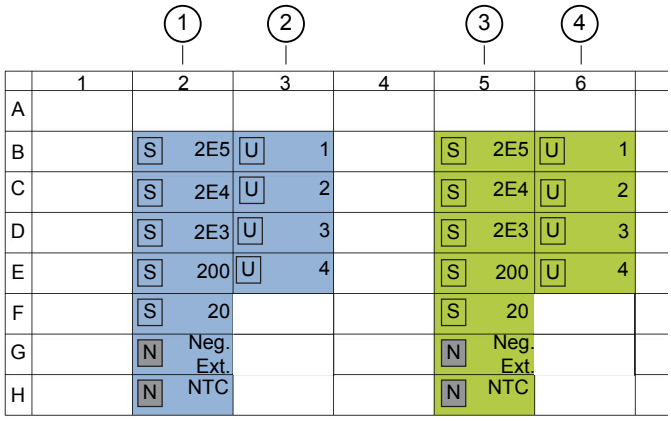

Figure 2 Example plate layout

In this example, each PCR series includes four unknown samples (U) and the recommended dilutions of P35S Standard (S). Negative extraction control and no-template control reactions (NTC; N) are also included.

① Standard dilutions and negative controls, P35S PCR

② Unknown samples, P35S PCR

③ Standard dilutions and negative controls, MSS PCR

④ Unknown samples, MSS PCR

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: FAM™ dye
   - TaqMan™ probe quencher: 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

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

Analyze results

See Figure 3 and Figure 4 for example data.

1. Confirm that results for the negative controls and P35S Standard are as expected.

Sample type	P35S PCR series	MSS PCR series
Negative extraction control or no-template control	No amplification[1]	No amplification [1]
P35S Standard dilution series	Amplification should be detected in all five dilutions for both P35S and MSS PCR series (see Figure 3). The standard curves derived from the dilution series should meet the following requirements (see Figure 4): • The efficiency of the curve should be 86–110% (slope between –3.7 and –3.1). • The correlation coefficient (R^2) should be >0.98.	

[1] Amplification in a negative control indicates contamination. The assay should be repeated.

2. Determine whether the P35S and MSS targets can be detected and quantified for each unknown sample.

Table 2 Quantifiability of samples

Unknown sample result	Interpretation
No amplification detected.	Not detected.
Amplification detected and the sample C_T falls within the corresponding standard curve.	Detected and quantifiable.
Amplification detected and the sample C_T is greater than the highest C_T of the corresponding standard curve.	Detected but not quantifiable. The concentration of DNA in the sample is too low.
Amplification detected and the sample C_T is lower than the lowest C_T of the corresponding standard curve.	Detected but not quantifiable. The concentration of DNA in the sample is too high. See "Input DNA requirements" on page 8.

Table 3 Interpretation of results

MSS PCR	P35S PCR	Interpretation
Quantifiable	Not detected	No P35S detected.
Quantifiable	Not quantifiable	The amount of P35S detected is lower than the limit of quantification.
Quantifiable	Quantifiable	The number of copies of P35S with respect to the number of copies of MSS can be calculated (step 3).
Not quantifiable	Not detected	No P35S detected and the amount of maize is lower than the limit of quantification.
Not quantifiable	Not quantifiable	The amounts of maize and P35S detected are lower than the limit of quantification.
Not detected	Not detected	No maize or P35S detected, or inhibitors are present. See "Test for the presence of inhibitors in the sample" on page 14.

3. If the results for both PCR series for the unknown samples are quantifiable, calculate the percentage of P35S as follows:
   a. Calculate the number of copies of P35S and MSS using the respective standard curves (Figure 4).
   b. Calculate the percentage of P35S:

$$\% \text{ P35S} = \frac{\text{No. of copies of P35S} \times 100}{\text{No. of copies of MSS}}$$

Your instrument software may be set up to automatically perform this calculation.

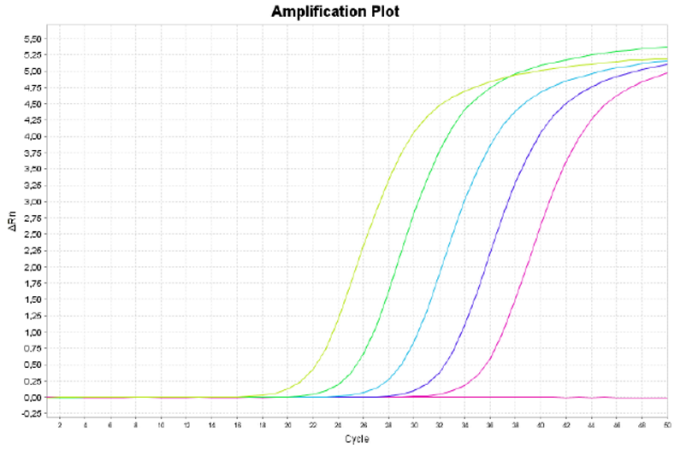

Figure 3 Example amplification curves for P35S Standard dilution series.

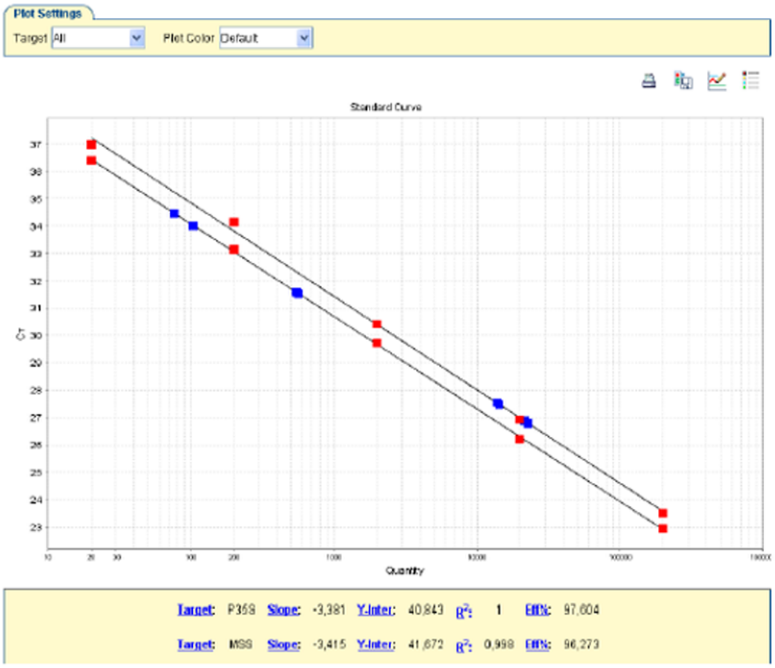

Figure 4 Example standard curves for P35S and MSS targets using P35S Standard dilution series.

Test for the presence of inhibitors in the sample

In this experiment, PCR amplification of the P35S Standard in the presence and absence of a sample that is suspected of containing PCR inhibitors is compared.

Set up and run two PCRs:

Component	Positive control	Test
Maize Master Mix (red disc)	7.5 µL	7.5 µL
General Master Mix (white disc)	12.5 µL	12.5 µL
P35S Standard, 1:10 dilution (4000 copies/µL)	1 µL	1 µL
Nuclease-free water	5 µL	—
Test sample	—	5 µL

The sample does not contain inhibitors if the amplification of both PCR reactions is similar.

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.

B

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

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  - 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.

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