

foodproof®

# GMO 35S Maize Quantification Kit

## Ready Reference Guide

Revision A, December 2023

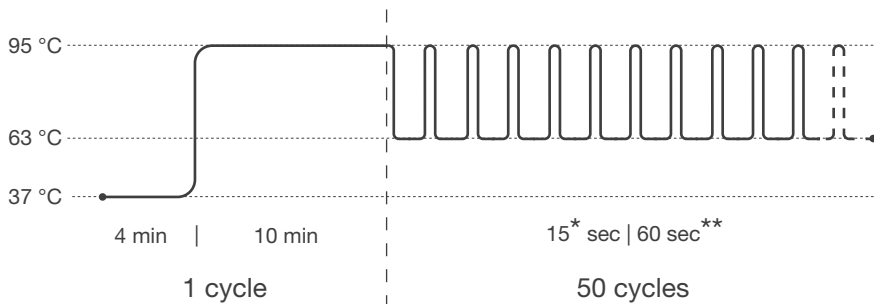
Product No. KIT230052

Before starting, it is strongly recommended to read the entire product manual available on our website.

### PROGRAM SETUP

Program your real-time PCR instrument before setting up the PCR reactions. Select the following channels:

- ▶ FAM: 35S and Maize (*ivr1*).



**Pre-incubation: 1 cycle**  
 Step 1: 37 °C for 4 min  
 Step 2: 95 °C for 10 min  
**Amplification: 50 cycles**  
 Step 1 : 95 °C for 15\* sec  
 Step 2\*\* : 63 °C for 60 sec

\* Use 5 s for LightCycler® 480

\*\* Fluorescence detection

For some real-time PCR instruments the probe quencher as well as the usage of a passive reference dye has to be specified. This kit contains probes with a non-fluorescent "dark" quencher and no passive reference dye.

### PREPARATION OF STANDARD CURVE

Use Calibrator DNA (purple cap) and Dilution Buffer (blue cap) to prepare dilutions according to table below. For each dilution step, pipet 30 µL (60 µL for duplicates) Dilution Buffer into a new reaction tube. Transfer 10 µL (20 µL for duplicates) from preceding step to new dilution step. Mix well between pipetting steps.

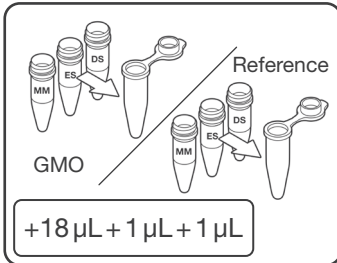
The prepared dilutions can be used for both standard curves, GMO gene and reference gene.

A typical experiment consists of 16 wells needed for standards and controls, plus 2 × n wells (n = number of food samples). Since a multiwell plate has 96 wells, 40 food samples can be analyzed during one PCR run if the GMO gene and the reference gene are analyzed in the same run. Some real-time PCR instruments provide the opportunity to import external standard curves generated in a previous run, then 46 food samples can be analyzed during one PCR run.

Dilution Step	Dilution Factor	Final Concentration
1	Undiluted	100
2	1:4	25
3	1:16	6.25
4	1:64	1.56
5	1:256	0.39
6	1:1024	0.098

# PREPARATION OF THE PCR MIX

Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves. Thaw reagents, mix (do not vortex!), and briefly spin vials before opening. For data interpretation and calculation please refer to the entire product manual.

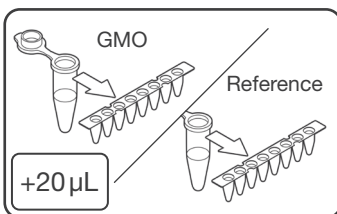


## 1. PREPARE PCR MIXES

**GMO PCR mix:** Add 18 µL of Master Mix (yellow cap), 1 µL of Enzyme Solution (red cap) and 1 µL of Dye Solution (black cap) for each reaction to a suitable tube.

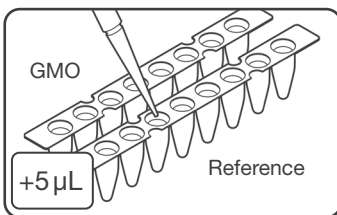
**Reference PCR mix:** Add 18 µL of Master Mix (green cap), 1 µL of Enzyme Solution (red cap) and 1 µL of Dye Solution (black cap) for each reaction to a suitable tube.

(n samples + 2 controls + at least one additional reaction to cover pipetting loss). Mix carefully but thoroughly by pipetting up and down.



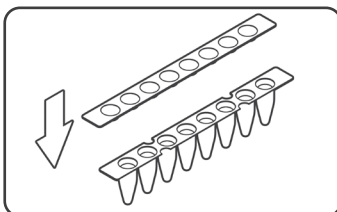
## 2. ADD PCR MIX

Pipet 20 µL of prepared GMO and Reference PCR mix into respective strip or plate well.



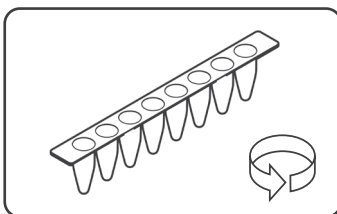
## 3. ADD SAMPLES AND CONTROLS

Sample DNA must be diluted at least 1:10 in the Dilution Buffer (blue cap). To each PCR mix (GMO and Reference), pipet 5 µL of samples, standards, Negative Control (colorless cap) or Control Template (purple cap) into respective wells.



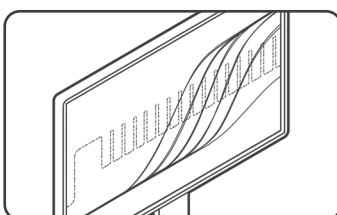
## 4. SEAL

Seal strips/plate accurately.



## 5. CENTRIFUGE

Briefly spin strips/plate in a suitable centrifuge.



## 6. START REAL-TIME PCR RUN

Cycle samples as described above.