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GMO Bt176 Maize Quantification Kit
Ready Reference Guide

Revision A, December 2023

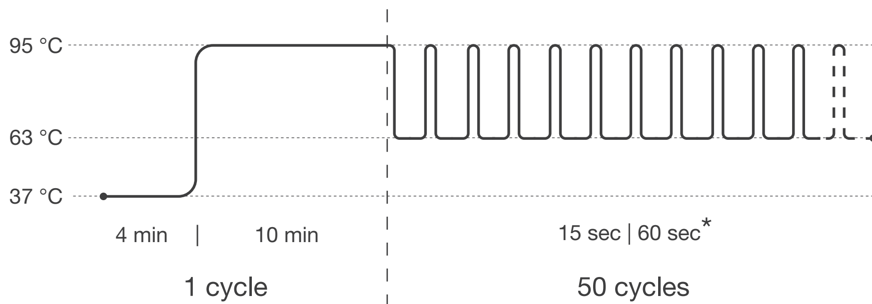
Product No. KIT230044

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: Bt176 and Maize (*zSSIIb*).



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

* Fluorescence detection

For some real-time PCR instruments the probe quencher as well as the use of a passive reference dye must be specified. This kit contains probes with TAMRA as quencher and no passive reference dye.

PREPARATION OF STANDARD CURVE

Use the Calibrator DNA (purple cap) and Dilution Buffer (blue cap) to prepare dilutions according to the table below. For each dilution step, pipet 30 µL (60 µL for duplicates) of 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

RR-KIT230044-RevA

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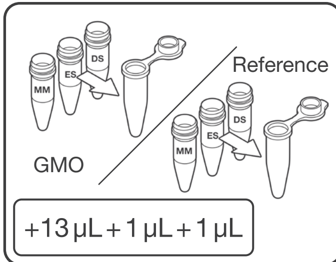
PREPARATION OF THE PCR MIX

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, refer to the entire product manual.



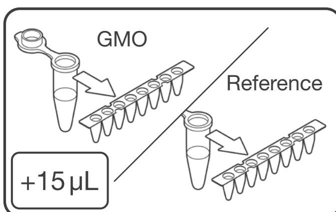
1. PREPARE PCR MIXES

GMO PCR mix: Add 13 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 13 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.

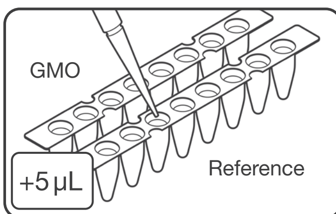
(n samples + 2 controls + at least one additional reaction to cover pipetting loss).

Mix carefully but thoroughly by pipetting up and down.



2. ADD PCR MIX

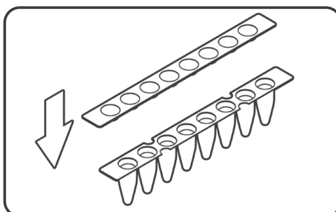
Pipet 15 μL of prepared GMO and Reference PCR mix into respective strip or plate well.



3. ADD SAMPLES AND CONTROLS

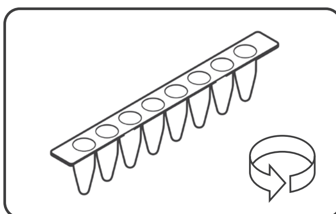
Sample DNA must be diluted at least 1:4 in the Dilution Buffer (blue cap).

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.



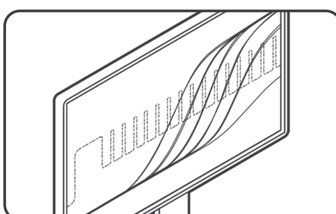
4. SEAL

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.

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