foodproof® SL

GMO Maize Multiplex Detection Kit (MON89034, CBH351, Bt176)

Ready Reference Guide

Revision A, December 2023

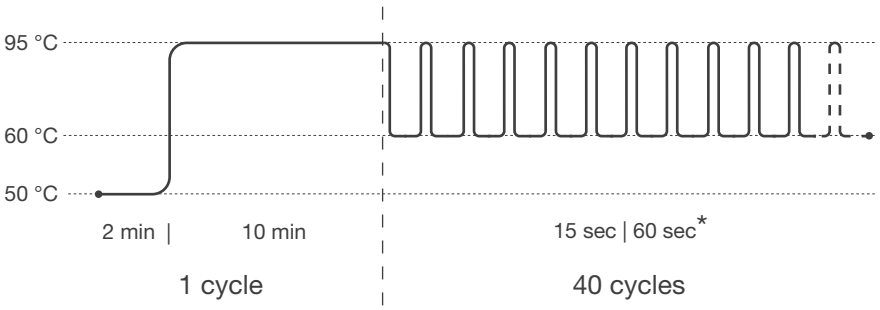
Product No. KIT230218

PCR kit for the qualitative detection of MON89034, CBH351 and Bt176 DNA using real-time PCR instruments. 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 (MON89034), VIC/HEX (CBH351), ROX (Bt176) and Cy5 (Internal Control).



Pre-incubation: 1 cycle
Step 1: 50 °C for 2 min
Step 2: 95 °C for 10 min
Amplification: 40 cycles
Step 1 : 95 °C for 15 sec
Step 2*: 60 °C for 60 sec

* 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.

DATA INTERPRETATION

Verify results of positive (Control Template) and negative controls (H_2O), before interpreting sample results. Always compare samples to positive and negative control. Review data from each channel and interpret results as described in the table.

FAM	VIC/HEX	ROX	Cy5	Result Interpretation
+	+	+	+ or -	Positive for MON89034, CBH351 and Bt176
-	+	+	+ or -	Positive for CBH351 and Bt176
+	-	+	+ or -	Positive for MON89034 and Bt176
+	+	-	+ or -	Positive for MON89034 and CBH351
-	+	-	+ or -	Positive for CBH351
+	-	-	+ or -	Positive for MON89034
-	-	+	+ or -	Positive for Bt176
-	-	-	+	Negative for MON89034, CBH351 and Bt176
-	-	-	-	Invalid

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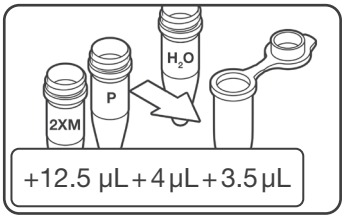
PREPARATION OF THE PCR MIX

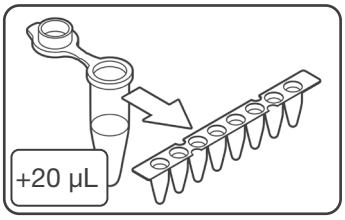
Take appropriate precautions to prevent contamination, e.g., by using filter tips and wearing gloves.
Thaw reagents, mix (do not vortex!) and briefly spin vials before opening.



1. PREPARE PCR MIX

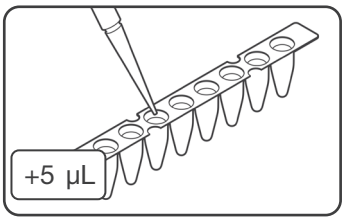
Add 12.5 µL Master Mix (2XM),
4.0 µL Primer/Probe Mix (P) and
3.5 µL PCR-grade H_2O (not included)
} for each reaction to a suitable tube.

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



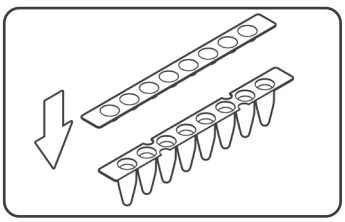
2. ADD PCR MIX

Pipette 20 µL of prepared PCR mix into each strip or plate well.



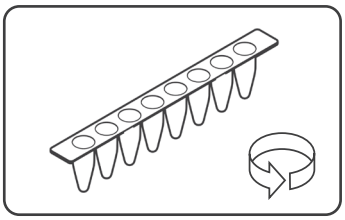
3. ADD SAMPLES AND CONTROLS

Pipette 5 µL of samples, negative control (PCR-grade H_2O) or Control Template (C) into respective wells.



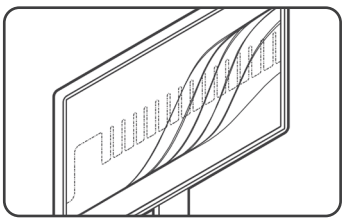
4. SEAL

Carefully seal strips/plate.



5. CENTRIFUGE

Briefly spin strips/plate in a suitable centrifuge.



6. START REAL-TIME PCR RUN

Cycle samples as described above.