foodproof® GMO RR Soya Quantification Kit

- Hybridization Probes (LC 1.x, 2.0, 480) - **Order No. R 300 19**

Quick Reference Procedure (for LC 1.x and 2.0)

Version 5, June 2013

A. LightCycler® Protocol

The following procedure is optimized for use with the LightCycler 1.x and 2.0 Systems. Program the LightCycler protocol before preparing the reaction mixes. A LightCycler protocol that uses the **food**proof GMO RR Soya Quantification Kit contains the following programs (for details on how to program the experimental protocol, see the LightCycler Operator's Manual):

Pre-incubation (activation of FastStart Taq DNA polymerase, denaturation of template DNA)			
Programs/Cycle Program Data	***Value***		
Cycles	1		
Analysis Mode	None		
Temperature Targets	***Segment 1***		
Target/Target Temperature [°C]	95		
Hold/Incubation Time [h:min:s]	00:10:00		
Ramp Rate/Temperature Transition Rate [°C/s]	20		
Sec Target/Secondary Target Temperature [°C]	0		
Step Size [°C]	0.0		
Step Delay [cycles]	0		
Acquisition Mode	None		
Amplification (of the target DNA)			
Programs/Cycle Program Data	***Value***		
Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target/Target Temperature [°C]	95	60	72
Hold/Incubation Time [h:min:s]	00:00:10	00:00:20	00:00:10
Ramp Rate/Temperature Transition Rate [°C/s]	20	20	20
Sec Target/Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0.0	0.0	0.0
Step Delay [cycles]	0	0	0
Acquisition Mode	None	Single	None

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Cooling (the rotor and thermal chamber)	
Programs/Cycle Program Data	***Value***
Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target/Target Temperature [°C]	40
Hold/Incubation Time [h:min:s]	00:00:30
Ramp Rate/Temperature Transition Rate [°C/s]	20
Sec Target/Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [cycles]	0
Acquisition Mode	None

B. Preparation of the Enzyme Master Mix

Prepare the **food**proof GMO RR Soya Enzyme Master Mix as described in the package insert in chapter 3.4.

C. Preparation of the PCR Mix

Proceed as described below for a 20 µl standard reaction.

Note: The master mixes for the Roundup Ready Soya- and the Reference Gene PCR must be set-up separately, using the respective Detection Mixes.

Important: Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.

Step	Action	
1	Depending on the total number of reactions, place the required number of LightCycler Capillaries in centrifuge adapters or in a LightCycler Sample Carousel in a LightCycler Centrifuge Bucket.	
2	• Thaw the solutions and, for maximal recovery of contents, briefly spin vials in a microcentrifuge before opening. • Mix carefully but thoroughly by pipetting up and down.	
3	In a 1.5 ml reaction tube, prepare the PCR Mix for one 20 µl reaction by adding the following components in the order mentioned below, then mixing gently but thoroughly by pipetting up and down.	
	Component	**Volume**
	H_2O PCR-grade (vial 7, colorless cap)	11 µl
	foodproof GMO RR Soya GMO Gene Detection Mix, 10× conc. (vial 1, yellow cap) or **food**proof GMO RR Soya Reference Gene Detection Mix, 10× conc. (vial 2, green cap)	2 µl
	foodproof GMO RR Soya Enzyme Master Mix. **Attention: Must be prepared by combining vials 3 and 4!**	2 µl
	Total volume	**15 µl**
	Note: To prepare the PCR Mix for more than one reaction, multiply the amount in the "Volume" column above by z, where z = the number of reactions to be run, + one additional reaction.	
4	• Mix carefully but thoroughly by pipetting up and down. Do not vortex. • Pipet 15 µl PCR mix into each LightCycler Capillary. • For the samples of interest, add 5 µl sample DNA to a capillary. Seal with a stopper. • For the negative control, add 5 µl PCR-grade H_2O (vial 7, colorless cap). Seal with a stopper. • Procedure A: For the external standards, add 5 µl of each dilution of **food**proof GMO RR Soya Calibrator DNA to the capillaries. Seal with a stopper. • Procedure B: For the positive control, add 5 µl **food**proof GMO RR Soya Calibrator DNA (vial 5, purple cap) to a capillary. Seal with a stopper.	
5	• Place the adapters, containing the capillaries, into a standard benchtop microcentrifuge. **Note:** Place the centrifuge adapters in a balanced arrangement within the centrifuge • Centrifuge at 700 × g for 5 s (3000 rpm in a standard benchtop microcentrifuge).	
6	Transfer the capillaries into the sample carousel of the LightCycler Instrument.	
7	Cycle the samples as described in section 3.2.	
8	• Procedure A: During the run, click on the Edit Samples button and enter the sample names in the loading screen. Define the positions of the dilutions of the **food**proof GMO RR Soya Calibrator DNA as "Standard" with the respective concentrations given in the table above. • Procedure B: For quantification with the LightCycler Relative Quantification Software, a specific loading scheme must be applied. Please refer to the Software Manual for further information.	

*__Note:__ For further information please refer to: www.bc-diagnostics.com/?cid=1237279134&lang=1

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