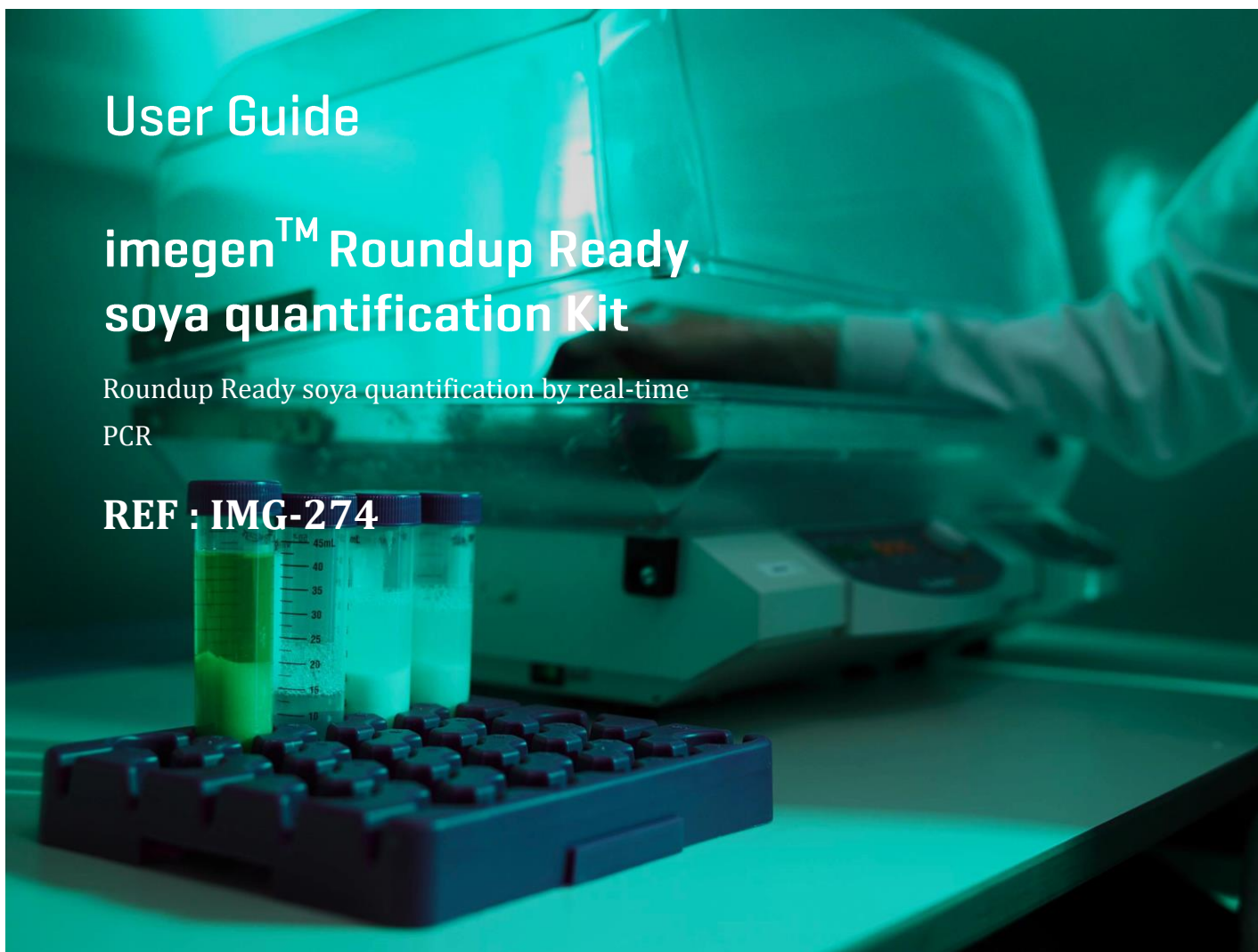


User Guide

imegenTM Roundup Ready[®] soya quantification Kit

Roundup Ready soya quantification by real-time
PCR

REF : IMG-274



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*All products sold by the imegen are subjected to rigorous quality control. The **imegenTM Roundup Ready soya quantification kit** has passed all internal validation tests, ensuring the reliability and reproducibility of each assay.*

For any questions about the applications of this product or its protocols, please contact our Technical Department:

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1. Product information

Kit description

Genetically modified organisms [GMOs] are widely distributed, with soy and corn being two of the most extensively cultivated crops worldwide. Indeed, Roundup Ready transgenic soy accounts for some 77% of the soy cultivated worldwide, thus making it the most widespread transgenic event and therefore the one that generates the most problems.

The European Union has established a legal framework to regulate the use, release into the environment and, above all, labelling of foodstuffs containing genetically modified organisms.

*The **imegen-Roundup Ready soya quantification Kit**, allows the percentage of Roundup Ready soy in a sample to be determined with respect to total soy.*

This kit uses Real-Time PCR technology and contains all the reagents required to quantify the Roundup Ready soy in DNA obtained from any food or feed. Furthermore, the kit contains the plasmid DNA used as a standard with which the samples can be compared to determine the percentage of Roundup Ready soy.

Content and storage of the kit

Sample analysis comprises two real-time PCR simultaneous processes:

- *One of them allows the total amount of soy DNA in the sample*
- *The other, allows the total amount of Roundup Ready soy DNA present in the sample*

To perform the reaction to determine the total soy DNA amount in the sample, this kit includes a master mix with two primers and a TaqMan®-type probe labelled with the FAM™ fluorophore. This reaction specifically amplifies an endogenous soy gene known as lectin.

To perform the reaction to determine the Roundup Ready soy DNA amount in the sample, this kit includes a master mix with two primers and a TaqMan®-type probe labelled with the FAM™ fluorophore. The reaction specifically amplifies the GTS-40-3-2 transgenic event in Roundup Ready soy. This fragment is event-specific, in other words it amplifies the binding region between the soy genome and the construct introduced in Roundup Ready soy.

imegen-Roundup Ready soya quantification Kit also includes a plasmid DNA standard containing a copy of each of the targets used during analysis. A comparison of the results obtained with the samples and this standard allows a relative quantification to be made and therefore the percentage of Roundup Ready soy with respect to the total soy present in the sample to be calculated.

The kit contains the necessary reagents to perform 48 reactions:

Reagents	Color	Amount	Storage
RR soy Master Mix	Green pad	360 µL	-20°C
Soy Master Mix	Purple pad	360 µL	-20°C
General Master Mix	White pad	2 x 600 µL	4°C
RR soy standard	Green cap	6 x 50 µL	-20°C

Table 1. Kit components and storage temperature of **imegen-Roundup Ready soya quantification Kit**

Equipment and material required but not supplied

In the following table the equipment and material requirements for using **imegen-Roundup Ready soya quantification Kit** are shown:

Equipment	
1	Real-time PCR Thermal Cycler with channels for detection of FAM™ (520 nm) and VIC® (550 nm)
2	Micropipettes (10 µl, 20 µl and 200 µl)
3	Table top centrifuge with adaptors for 96 well PCR plates and/or 0.2 ml tubes
4	Vortex

Materials	
1	Optical 96-well reaction plates or 0.2 ml optical tubes
2	Optical adhesive film for 96 well plates or optical adhesive covers for 0.2 ml tubes
3	Disposable micropipette filter tips
4	1.5 ml sterile tubes
5	Powder-free latex gloves

Detection and quantification limits

Limit of quantification: 20 copies of DNA

Detection limit of the PCR technique [soy system]: 3 copies of DNA

Detection limit of the PCR technique [Roundup Ready soy system]: 3 copies of DNA

This Kit allows relative quantifications of up to 0.01% of Roundup Ready soy to be determined with respect to total soy in a sample. The relative limit of quantification varies depending on the sample analysed.

2. Real time PCR

PCR reactions preparation

Two absolute quantifications are performed during the course of the relative quantification of Roundup Ready soy present in a sample. The first of these determines the total amount of soy present in the sample and the second determines the amount of Roundup Ready soy.

Preparation of the amplification reactions includes:

- Standard dilutions
- Negative PCR and/or extraction controls
- Sample analysis in duplicate

To estimate the amount of necessary reagents, we recommend make calculations taking into account the number of samples and controls to be simultaneously analysed, and then considering one more reaction, or increase a 10% the volume of each reagent.

The recommended protocol for preparation of reactions is showed below:

1. Thaw a vial of RR soy standard and prepare four 1:10 serial dilutions of this standard. This process results in the quantitative standards with which the samples can be compared.

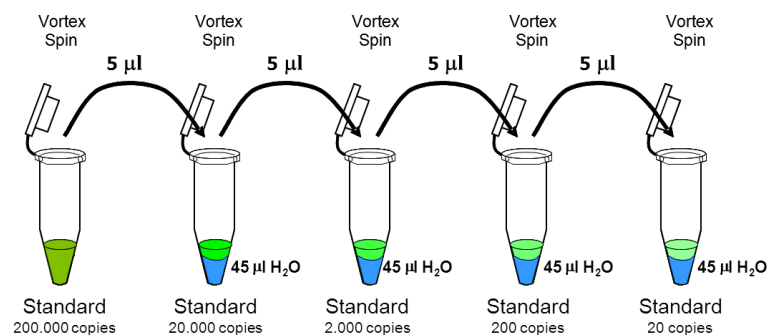


Figure 1: Four serial standard dilutions are made from RR soy Standard to perform two standard curves

2. Thaw the Master Mixes, negative controls and DNA samples [if stored frozen].
3. Shake each of the reagents on the vortex whilst keeping them cold.
4. Add into a 1.5 mL tube, the following reagents:

Reagents	Amount per reaction
Soy Master Mix	7.5 μ L
General Master Mix	12.5 μ L

Table 3. Reagents amount per reaction

Add into another 1.5 mL tube, the following reagents:

Reagents	Amount per reaction
RR soy Master Mix	7.5 μ L
General Master Mix	12.5 μ L

Table 4. Reagents amount per reaction

5. Vortex and spin the 1.5 mL tubes and dispense 20 μ L per well or tube of 0.2 ml [see the example for three samples in figure 2].
6. Add 5 μ L of each sample DNA [10–25 ng/ μ L] to the corresponding wells:
 - a. Total soy reactions and,
 - b. RR soy amplification reactions
7. Add 5 μ L of each standard dilution to the corresponding wells:
 - a. Total soy reactions and,
 - b. RR soy amplification reactions
8. Add 5 μ L of each control [negative control and DNA extraction control] to the corresponding wells:
 - a. Total soy reactions and,
 - b. RR soy amplification reactions
9. Seal the plate with optical film and spin.

10. Load the plate into a thermal cycler and then perform a run using the conditions showed in the next section.

	1	2	3	4	5	6	7	8
A								
B		S Soja 2E5	U Soja		S RR 2E5	U RR		
C		S Soja 2E4	U Soja		S RR 2E4	U RR		
D		S Soja 2E3	U Soja		S RR 2E3	U RR		
E		S Soja 200	U Soja		S RR 200	U RR		
F		S Soja 20	N Soja		S RR 20	N RR		

Soy Master Mix
RR soy Master Mix

Figure 2: Proposed design analysis for 2 samples obtained from the same DNA extraction round.

* We strongly recommend using an **extraction negative control** for each run of extractions carried out. This control consists in one tube to which no sample is added and which is submitted to the same extraction process as the other samples. Likewise, we recommended using a **PCR negative control** for each PCR run; this tube contains no DNA but all PCR reagents.

PCR amplification program

This kit is compatible with the Real-time PCR platforms 7500 FAST, StepOne Real-Time PCR System [Thermo Scientific], and any other Real-time PCR platforms equipped with FAM and VIC channels.

Probes	Receptor	Quencher
Soy-P	FAM TM	MGB
RR soy-P	FAM TM	MGB

Table 4. Probes information

The following instructions should be taken into account in order to setup the amplification program:

- Reaction volume: 25 μ L
- Targets: FAM and VIC
- In case the quencher has to be defined, select MGB for all probes. If the real time PCR system does not take into account the quenchers, select only the receptors [FAM and VIC].
- If the real time PCR system is a 7500 Fast or a StepOne Real-Time PCR system [Thermo Scientific] select *Quantitation- Standard curve* as a type of experiment and include *ROXTM* as a reference.
- Ramp rate: standard
- Optimal program:

Fields	Step 1 Enzyme activation	Step 2 PCR	
Cycle Number	1 initial cycle	50 cycles	
		Denaturation	Primers binding/Extention
Temperature	95°C	95°C	60°C
Time	10 minutes	15 seconds	1 minute*

Table 5. Optimal PCR program



*Fluorescence detection

Note: This program has been validated on a StepOne Real-Time PCR System from Applied Biosystems. If you use another brand or model of thermal cycler, you may need the amplification program to be adjusted. Please contact our service department for advice.

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3. Results analysis

Before analysing the sample results, it should be confirmed that the results obtained with the different controls are as expected:

- **Negative controls:** No amplification should be detected in either the reaction corresponding to soy or that corresponding to Roundup Ready soy. Amplification in a negative control indicates the presence of contamination and therefore that the assay should be repeated.
- **RR soy standard:** Amplification should be detected for the five points corresponding to the soy standard and the five points corresponding to the Roundup Ready soy standard. Furthermore, the curves obtained using the standard points should meet the following requirements:

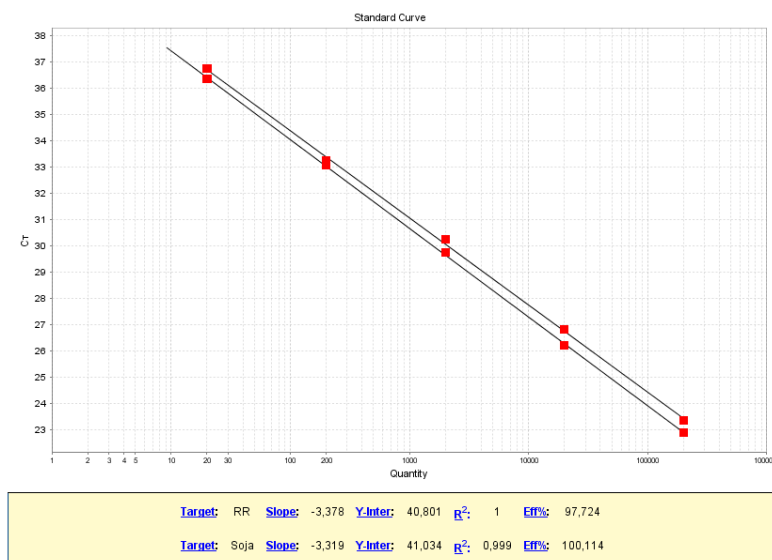


Figure 3: Standard curves for total soy and RR soy targets. Red dots represent the dilutions of the standard.

Furthermore, the curves obtained using the standard points should meet the following requirements:

- The efficiency of the curve should be between 80% and 110%.
- The slope of the curve should be between -3.1 and -3.9.
- The correlation coefficient [R^2] should be greater than 0.98.

Once the controls have been verified, the results obtained with the samples can be analysed. If duplicated have been performed, the results for both replicates should be similar.

Three results are possible for each amplification reaction of both soy and Roundup Ready soy:

- **Not detected:** No amplification in the sample. The amplification curve is flat.
- **Not quantifiable:** Amplification is detected in the sample but to an extent lower than the last point on the curve. When the Ct for the sample is greater than the Ct for the 20-copy standard, it can be concluded that the analyte is present in the sample but is not quantifiable.
- **Quantifiable:** Amplification is detected in the sample to an extent greater than the last point on the curve. When the amplification Ct for the sample is interpolated between the values for the standard points, the quantitative result can be considered to be reliable and can be used to calculate the percentage of Roundup Ready soy.

The following formula should be used to calculate the percentage of Roundup Ready soy with respect to total soy present in the sample:

$$\% \text{ RR Soy} = \frac{\text{No. of copies of RR soy} \times 100}{\text{No. of soy copies}}$$

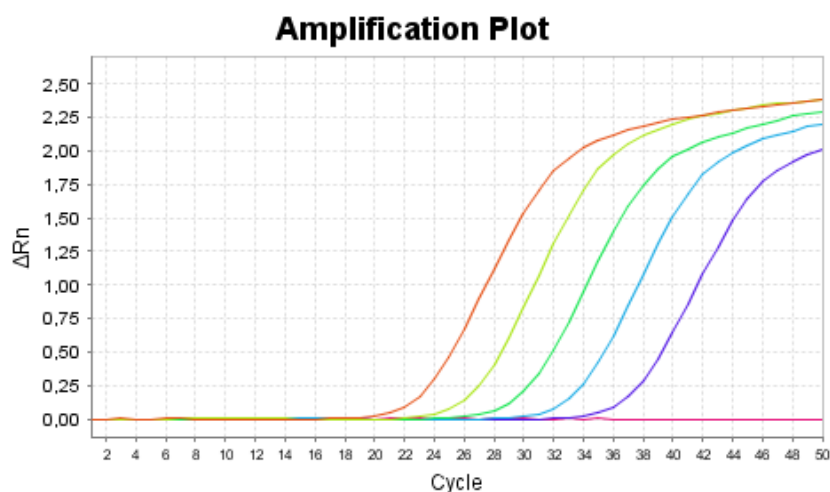


Figure 4: Amplification curves for each of the dilutions of the RR soy Standard using RR soy master mix

The following table shows the interpretation that should be done from the obtained result:

Soy	RR soy	Interpretation
Quantifiable	Not detected	No Roundup Ready soy detected in the sample
Quantifiable	Not quantifiable	The amount of Roundup Ready soy detected in the sample is lower than the limit of quantification
Quantifiable	Quantifiable	The amount of Roundup Ready soy with respect to total soy in the sample is X%
Not quantifiable	Not detected	No Roundup Ready soy detected in the sample, the amount of soy present in the sample is lower than the limit of quantification
Not quantifiable	Not quantifiable	The amounts of soy and Roundup Ready soy detected in the sample are lower than the limit of quantification
Not detected	Not detected	No soy or Roundup Ready soy detected in the sample*

Table 8. Possible results and their interpretation

** It is possible that the inability to detect soy DNA in a sample is due to the presence of inhibitors in the DNA used. To check for the absence of inhibitors in the sample, we recommend that you use an inhibition control consisting of amplification with Soy Master Mix in a well containing test sample DNA together with 1 μ L of the inhibition control corresponding to the dilution containing 20,000 copies of the standard. Another well containing 5 μ L of water and 1 μ L of the same inhibition control should be amplified in parallel. If the amplification of both reactions is similar, it can be concluded that the sample is not inhibited.*