

## PRODUCT INFORMATION

# Thermo Scientific ABsolute Blue qPCR SYBR Green ROX Mix

#AB-4163/A 5 mL

Lot \_ Expiry Date \_

## Ordering Information

Component	#AB-4162/B 1600 rxns of 25 µL	#AB-4163/A 400 rxns of 25 µL
2X ABsolute Blue SYBR Green ROX Mix	16 × 1.25 mL	5 mL
1 M MgCl <sub>2</sub>	100 µL	100 µL

Store at -20°C



[www.thermoscientific.com/onebio](http://www.thermoscientific.com/onebio)

## Description

Thermo Scientific ABsolute Blue qPCR SYBR® Green ROX Mix has been developed to quantify DNA and cDNA. With the exception of primers and template, this 2X mix contains all the components required to perform a rapid, sensitive and reproducible qPCR reaction:

- Thermo Scientific Thermo-Start DNA Polymerase, a chemically modified hot-start version of Thermo Scientific ThermoPrime DNA Polymerase, which prevents non-specific amplification during the reaction set-up. Thermo-Start™ has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading). **This enzyme requires an activation step at 95°C for 15 minutes.**
- Proprietary reaction buffer which provides highly sensitive, specific and consistent fluorescence readings for real-time and end-point analysis. This buffer has been optimized for MgCl<sub>2</sub> and enhancers to improve amplification across a wide range of templates including plant DNA and GC rich fragments. It contains an inert blue dye to assist in the visualization of the Absolute™ Blue SYBR Green ROX after aliquoting into the reaction well.
- dNTP's, including dTTP to improve reaction sensitivity and efficiency compared to dUTP.
- SYBR Green I, a dye which fluoresces after binding of the double-stranded DNA. The overall fluorescence increases proportionally to the double-stranded DNA concentration.
- ROX, passive reference dye for normalization of data.

## Cycler Compatibility

ABsolute Blue qPCR SYBR Green ROX Mix is compatible for use with qPCR cyclers requiring high ROX dye level, including ABI PRISM® 7000, 7300, 7700, 7900 and 7900HT (including Fast-Block).

## Blue Dye

This proprietary inert blue dye allows quick and easy visualization of the amount of the mix in the well, minimizing aliquoting errors. It does not interfere with the qPCR reaction and is only available in master mix format.

## ROX Dye

ROX is an internal passive reference dye used to normalize the fluorescent reporter signal generated in qPCR. The concentration of ROX in the final 1X reaction is 500 nM.

## MgCl<sub>2</sub>

The initial concentration of MgCl<sub>2</sub> in the ABsolute Blue SYBR Green ROX Mix corresponds to 3 mM in the final 1X reaction. This concentration is effective over a broad range of templates. Some assays may be improved further with MgCl<sub>2</sub> optimization. A separate vial of 1 M MgCl<sub>2</sub> is therefore supplied with each kit. MgCl<sub>2</sub> concentration can be increased as follows: each 2.5 µL or 10 µL addition of MgCl<sub>2</sub> to the 1.25 mL or 5 mL undiluted ABsolute Blue SYBR Green ROX Mix respectively corresponds to an increase of 1 mM in the final 1X reaction. Scale up or down accordingly. Mix thoroughly by inverting the vial ten to twenty times. **Do not vortex.**

## Storage Conditions

Store at -20°C until ready for use. ABsolute Blue qPCR SYBR Green ROX Mix is stable for a minimum of 12 months. The reagents can be stored at 4°C for up to 1 month. Avoid repeated freeze thawing. The ROX and SYBR Green dyes are light sensitive; exposure should be minimized.

## Additional Info

The use of disposable gloves, DNase and RNase free filter tips and plastics is recommended.

For optimal results, the recommended amplicon length is in the range of 60 to 300 bp.

As best performance is achieved with dTTP, the ABsolute Blue SYBR Green ROX Mix contains a nucleotide mix with dTTP instead of dUTP.

## Protocol

Thaw the reagents on ice. Mix and spin down the solutions before use to recover the maximum amount. **Do not vortex the ABsolute Blue SYBR Green ROX.**

Briefly centrifuge to avoid bubbles within the wells, as these will interfere with the fluorescence. Always include a no template control (NTC).

Example of Reaction Mix preparation for a 25 µL final reaction:

	Volume	Final Concentration
<b>2X ABsolute Blue SYBR Green ROX</b>	12.5 µL	1X
<b>Forward primer (1 µM)*</b>	1.75 µL	70 nM
<b>Reverse primer (1 µM)*</b>	1.75 µL	70 nM
<b>Template (DNA or cDNA)**</b>	1-5 µL	< 250 ng/rxn
<b>Water, nuclease-free (#R0581)</b>	To 25 µL	
<b>Total volume</b>	25 µL	

\*For optimization, a primer titration should be performed from 50 nM to 300 nM final concentration. Scale up or down the volume and concentration as appropriate.

\*\* The volume of template to add to the qPCR reaction can be adjusted as required. For standard templates only 1 µL should be added to reduce carryover of PCR inhibitors. This volume can be increased up to 5 µL for low copy number templates.

Example of qPCR thermal cycling program:

	Temp.	Time	Number of cycles
<b>Enzyme activation</b>	95°C	15 min	1 cycle
<b>Denaturation</b>	95°C	15 s	40 cycles
<b>Annealing*</b>	50-60°C	30 s	
<b>Extension**</b>	72°C	30 s	

\*Annealing temperature dependent on primer sequence.

\*\*Time of extension depends on the length of the amplicon. If the amplicon exceeds 300 bp amplification time should be adapted (Thermo-Start DNA Polymerase extends approximately at 1000 bp/min).

It is recommended to perform a melt curve to confirm the specificity of the reaction. Example of a **melt curve program\***:

<b>Denaturation</b>	95°C	30 s	1 cycle
<b>Starting temp.</b>	60°C	30 s	1 cycle
<b>Melting step**</b>	60°C	10 s	80 cycles

\*Melt curve program may vary depending on instrument manufacturer and software.

\*\*Increase set point temperature by 0.5°C per cycle.

## CERTIFICATE OF ANALYSIS

ABsolute Blue qPCR SYBR Green ROX Mix is tested functionally using qPCR. The product must demonstrate linearity of amplification over a specified serial dilution of human genomic DNA.

Quality authorized by:

 Jurgita Zilinskiene

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