

**PRODUCT INFORMATION**

**Thermo Scientific  
ABsolute qPCR SYBR Green Mix**

**#AB-1158/B**      16 x 1.25 mL

**Lot \_**              **Expiry Date \_**

**Ordering Information**

Component	#AB-1158/B 1600 rxns of 25 µL	#AB-1159/A 400 rxns of 25 µL
2X ABsolute qPCR SYBR Green 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 qPCR SYBR® Green 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 *Taq* 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.
- 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.

## MgCl<sub>2</sub>

The initial concentration of MgCl<sub>2</sub> in the ABsolute qPCR SYBR Green 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 addition of MgCl<sub>2</sub> to the 1.25 mL undiluted ABsolute qPCR SYBR Green 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.**

## Cycler Compatibility

ABsolute™ qPCR SYBR Green Mix is compatible with all qPCR cyclers that do not require a reference dye. For an exhaustive list, please refer to our latest catalog or contact our Tech Support team.

## Storage Conditions

Store at -20 °C until ready for use. The reagents can be stored at 4 °C for up to 1 month. Avoid repeated freeze thawing. The SYBR Green dye is 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 qPCR SYBR Green Mix contains a nucleotide mix with dTTP instead of dUTP.

## Protocol

Thaw the reagents on ice, mix the solutions and spin down before use to recover the maximum amount. Do not vortex the ABSolute qPCR SYBR Green Mix. 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 qPCR SYBR Green Mix</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 depends 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 qPCR SYBR Green 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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