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PRODUCT INFORMATION

Thermo Scientific Absolute SYBR Green Low ROX qPCR Mix

#AB-1323/A 5 mL

Lot _ **Expiry Date _**

Ordering Information

Component	#AB-1322/B 1,600 x 25 µL rxns	#AB-1323/A 400 rxns of 25 µL
2X ABsolute SYBR Green Low ROX qPCR Mix	16 x 1.25 mL (green)	5 mL
$MgCl_2$ (1 M)	100 µL (clear)	100 µL

Store at -20°C

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Description

Thermo Scientific Absolute SYBR® Green Low ROX qPCR 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 $MgCl_2$ 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.
- ROX, passive reference dye for normalization of data.

Cycler Compatibility

ABsolute™ SYBR Green Low ROX qPCR Mix is compatible for use with any qPCR cyclers requiring low ROX dye levels, including ABI PRISM® 7500 (including Fast-Block) and Stratagene Mx4000®, Mx3000P®, Mx3005P™.

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 25 nM.

$MgCl_2$

The initial concentration of $MgCl_2$ in the ABsolute SYBR Green Low qPCR 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 $MgCl_2$ optimization. A separate vial of 1 M $MgCl_2$ is therefore supplied with each kit.
$MgCl_2$ concentration can be increased as follows: each 2.5 µl or 10 µl addition of $MgCl_2$ to the 1.25 ml or 5 ml undiluted ABsolute SYBR Green Low qPCR 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 SYBR Green Low qPCR 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 SYBR Green Low ROX qPCR 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 Low ROX qPCR 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:

Reaction Mix	Volume	Final Concentration
2X ABsolute SYBR Green Low ROX qPCR Mix	12.5 µL	1X
Forward primer (1 µM)*	1.75 µL	70 nM
Reverse primer (1 µM)*	1.75 µL	70 nM
Template (DNA or cDNA)**	1-5 µL	< 250 ng/rxn
Water, nuclease-free(#R0581)	To 25 µL	
Total volume	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 the carryover of any PCR inhibitor. 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
Enzyme activation	95 °C	15 min	1 cycle
Denaturation	95 °C	15 s	40 cycles
Anealing*	50-60 °C	30 s	
Extension**	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*:

Denaturation	95 °C	30 s	1 cycle
Starting temp.	60 °C	30 s	1 cycle
Melting step**	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 SYBR Green Low ROX qPCR 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

TECHNICAL SUPPORT:

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Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.