

Thermo Scientific Absolute QPCR SYBR Green Mix

Description

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-Start™ DNA Polymerase, a chemically modified hot-start version of ThermoPrime *Taq* DNA Polymerase, which prevents non-specific amplification during the reaction set-up. **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 MgCl₂ 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.

Kit Contents

Vial	Pack Size (cap color)	
	A	B
Absolute QPCR SYBR Green Mix (2X)	2 x 1.25 ml (green)	16 x 1.25 ml (green)
MgCl ₂ (1 M)	100 µl (clear)	100 µl (clear)

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 (see page 4).

* For RNA template, use Verso™ SYBR® Green I-Step QRT-PCR Kit Plus ROX Vial (AB-4104)

INFORMATION

Thermo-Start™ DNA Polymerase

The enzyme requires an activation step at 95°C for 15 minutes.

Thermo-Start™ has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading).

MgCl₂

The initial concentration of MgCl₂ 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 MgCl₂ optimization. A separate vial of 1 M MgCl₂ is therefore supplied with each kit.

MgCl₂ concentration can be increased as follows: each 2.5 µl or 10 µl addition of MgCl₂ to the 1.25 ml or 5 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.**

Storage Conditions

Store at -20°C until ready for use. ABsolute™ QPCR SYBR® Green 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 SYBR® Green dye is light sensitive; exposure should be minimized. Shipped on ice within the UK and on dry ice internationally and within the US.

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.

Tips before use

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).

PROTOCOL

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

	Volume	Final Concentration
Reaction Mix		
Absolute QPCR SYBR Green Mix (2X)	12.5 µl	1X
Forward primer (1 µM) ^a	1.75 µl	70 nM
Reverse primer (1 µM) ^a	1.75 µl	70 nM
Water (PCR grade) ^b	variable	
Template (DNA or cDNA) ^c	1 - 5 µl	<250 ng/reaction
Total volume	25 µl	

Example of a qPCR thermal cycling program:

	Temp.	Time	Number of cycle
Enzyme activation	95°C	15 min	1 cycle
Denaturation	95°C	15 sec	40 cycles
Annealing ^d	50-60°C	30 sec	
Extension ^e	72°C	30 sec	

It is recommended to perform a melt curve to confirm the specificity of the reaction.

Example of a melt curve program^f:

Denaturation	95°C	30 sec	1 cycle
Starting temp.	60°C	30 sec	1 cycle
Melting step ^g	60°C	10 sec	80 cycles

Notes

- a – 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.
- b – The volume of the total reaction should be completed up to 25 µl with water.
- c – 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.
- d – Annealing temperature dependent on primer sequence.
- e – 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).
- f – Melt curve program may vary depending on instrument manufacturer and software.
- g – Increase set point temperature by 0.5°C per cycle.

Quality control

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.

Ordering Information

AB-1158/A	ABsolute™ QPCR SYBR® Green Mix	200 x 25 µl rxns
AB-1158/B	ABsolute™ QPCR SYBR® Green Mix	1,600 x 25 µl rxns
AB-1159/A	ABsolute™ QPCR SYBR® Green Mix	400 x 25 µl rxns
AB-1159/B	ABsolute™ QPCR SYBR® Green Mix	4,000 x 25 µl rxns

All formats are supplied with an additional vial of 1 M MgCl₂.

Related Products

Cat. No.	Description	Quantity
AB-4166/A	ABsolute™ Blue QPCR SYBR® Green Mix Plus ROX Vial	2 x 1.25 ml
AB-0600/W	ABgene 96-Well PCR Plate (Non-Skirted, white) *	25 plates
AB-0800/W	ABgene 96-Well PCR Plate (Skirted, white) *	25 plates
AB-0900/W	ABgene 96-Well PCR Plate (Segmented, Semi-Skirted, white) *	25 plates
AB-1170	ABsolute™ QPCR Adhesive Seal	50 sheets
AB-0866	Ultra Clear Cap Strips (8 caps)	120 strips

* For cyclor compatibility and other color choices, see our latest catalog or visit www.thermo.com/abgene

For technical information or troubleshooting contact Thermo Scientific Genomics Tech Support:

Troubleshooting:	Email	Phone
North America (US, Canada, Central/South America)	Techservice.genomics@thermofisher.com	+1 (800) 235-9880
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