

## Thermo Scientific ABsolute QPCR SYBR Green Fluorescein Mix

### Description

ABsolute™ QPCR SYBR® Green Fluorescein 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 Plus 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 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 to the double-stranded DNA. The overall fluorescence increases proportionally to the double-stranded DNA concentration.
- Fluorescein, passive reference dye for normalization of data.

### Kit Contents

Vial	Pack Size (cap color)	
	A	B
ABsolute SYBR Green Fluorescein (2X)	5ml (clear)	10 x 5ml (clear)
MgCl <sub>2</sub> (1 M)	100µl (clear)	3 x 100µl (clear)

### Cycler Compatibility

ABsolute™ QPCR SYBR® Green Fluorescein Mix is compatible with all QPCR cyclers that require fluorescein including the Bio-Rad iCycler® and MyiQ™.

\* For RNA template, use Verso™ SYBR® Green 1-Step QRT-PCR Fluorescein Kit (AB-4107)

## 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).

**Fluorescein Dye**

Fluorescein acts as a passive reference dye to facilitate normalization of data. The concentration of fluorescein in the ABsolute QPCR SYBR Green Fluorescein Mix corresponds to 10 nM in the final 1X reaction.

**MgCl<sub>2</sub>**

The initial concentration of MgCl<sub>2</sub> in the ABsolute QPCR SYBR Green Fluorescein 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 QPCR SYBR Green Fluorescein 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 Fluorescein 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 fluorescein and SYBR® Green dyes are light sensitive; exposure should be minimized. Shipped on ice within the UK and on dry ice for international 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 Fluorescein Mix contains a nucleotide mix with dTTP instead of dUTP.

DIRECTIONS FOR USE

**Tips and Protocol**

Thaw the reagents on ice. Mix and spin down the solutions before use to recover the maximum amount. **Do not vortex the Absolute QPCR SYBR Green Fluorescein 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
Reaction Mix		
Absolute QPCR SYBR Green Fluorescein Mix (2X)	12.5 µl	1X
Forward primer (1 µM) <sup>a</sup>	1.75 µl	70 nM
Reverse primer (1 µM) <sup>a</sup>	1.75 µl	70 nM
Water (PCR grade) <sup>b</sup>	variable	
Template (DNA or cDNA) <sup>c</sup>	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	<b>15 min</b>	1 cycle
Denaturation	95°C	15 sec	40 cycles
Annealing <sup>d</sup>	50-60°C	30 sec	
Extension <sup>e</sup>	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**<sup>f</sup>:

Denaturation	95°C	30 sec	1 cycle
Starting temp.	60°C	30 sec	1 cycle
Melting step <sup>g</sup>	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 carryover of PCR inhibitors. 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 Fluorescein 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-1219/A	ABsolute™ QPCR SYBR® Green Fluorescein Mix	200 x 25 µl rxns
AB-1219/B	ABsolute™ QPCR SYBR® Green Fluorescein Mix	1,600 x 25 µl rxns
AB-1220/A	ABsolute™ QPCR SYBR® Green Fluorescein Mix	400 x 25 µl rxns
AB-1220/B	ABsolute™ QPCR SYBR® Green Fluorescein Mix	4,000 x 25 µl rxns

All formats are supplied with an additional vial of 1 M MgCl<sub>2</sub>.

### Related Products

Cat. No.	Description	Quantity
AB-4219/A	ABsolute™ Blue QPCR SYBR® Green Fluorescein Mix	2 x 1.25 ml
AB-0900	Thermo-Fast™ 96 Semi-Skirted PCR Plate, natural *	25 plates
AB-0900/W	Thermo-Fast™ 96 Semi-Skirted PCR Plate, white *	25 plates
AB-1170	ABsolute™ QPCR Seal (adhesive seal)	50 sheets
AB-0812	Clear Seal Diamond (heat seal)	100 sheets
AB-0866	Ultra Clear Cap Strips (8 caps)	120 strips

\* For Cycler compatibility and other color choices, see our latest catalogue or visit [www.abgene.com](http://www.abgene.com)

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

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