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Thermo Scientific ABsolute Blue QPCR SYBR Green Fluorescein Mix

Description

ABsolute™ Blue 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 $MgCl_2$ 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 Fluorescein Mix 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 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 Blue SYBR Green Fluor (2X)	2 x 1.25ml (green)	16 x 1.25ml (green)
$MgCl_2$ (1 M)	100µl (clear)	100µl (clear)

Cycler Compatibility

ABsolute™ Blue 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)

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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).

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.

Fluorescein Dye

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

$MgCl_2$

The initial concentration of $MgCl_2$ in the ABsolute Blue 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 $MgCl_2$ 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 Blue 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™ Blue 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 Blue 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 Blue SYBR Green Fluor.** 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 Blue SYBR Green Fluor (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 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.

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Quality control

ABsolute Blue 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-4219/A	ABsolute™ Blue QPCR SYBR® Green Fluorescein Mix	200 x 25 µl rxns
AB-4219/B	ABsolute™ Blue QPCR SYBR® Green Fluorescein Mix	1,600 x 25 µl rxns
AB-4220/A	ABsolute™ Blue QPCR SYBR® Green Fluorescein Mix	400 x 25 µl rxns
AB-4220/B	ABsolute™ Blue QPCR SYBR® Green Fluorescein Mix	4,000 x 25 µl rxns

All formats are supplied with an additional vial of 1 M $MgCl_2$.

Related Products

Cat. No.	Description	Quantity
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

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Literature Code: AB-4219-v6-0411