Thermo SCIENTIFIC

Thermo Scientific ABsolute Blue QPCR SYBR Green Low ROX Mix

Description

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

Kit Contents

Vial	Pack Size (cap color)		
	A	B	D
Blue QPCR SYBR Low ROX (2X)	5ml (clear)	10 x 5ml (clear)	50ml (clear)
$MgCl_2$ (1 M)	100µl (clear)	3 x 100µl (clear)	3 x 100µl (clear)

Cycler Compatibility

ABsolute™ Blue QPCR SYBR® Green Low ROX 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™.

* **For RNA template**, use Verso SYBR® Green 1-Step QRT-PCR Low ROX Kit (AB-4106)

Thermo SCIENTIFIC

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.

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 Blue QPCR SYBR Green Low 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 Blue QPCR SYBR Low 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™ Blue QPCR SYBR® Green Low 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. 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 QPCR SYBR Green Low ROX 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 QPCR SYBR Green Low ROX 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	Blue QPCR SYBR Low ROX (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 Blue QPCR SYBR Green Low ROX 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-4322/A	ABsolute™ Blue QPCR SYBR® Green Low ROX Mix	200 x 25 µl rxns
AB-4322/B	ABsolute™ Blue QPCR SYBR® Green Low ROX Mix	1,600 x 25 µl rxns
AB-4323/A	ABsolute™ Blue QPCR SYBR® Green Low ROX Mix	400 x 25 µl rxns
AB-4323/B	ABsolute™ Blue QPCR SYBR® Green Low ROX 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-0600/W	Thermo-Fast™ 96 Non-Skirted, white *	25 plates
AB-1100/W	Thermo-Fast™ 96 PCR Detection Plate, white *	25 plates
AB-1400/W	Thermo-Fast™ 96 PCR Detection Plate Mark II, 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

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
Europe (EU, Middle East, Africa)	Techservice.emea.genomics @thermofisher.com	(+) 44 1372 840410
Other Countries	www.thermoscientific.com/ dharmacondistributors	

conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer, where such research does not include testing, analysis or screening services for any third party in return for compensation on a per test basis. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes , Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300, Fax: (541) 335-0354.

The purchase of this product includes a limited, nontransferable license, under specific claims of one or more U.S. patents owned by the University of Utah Research Foundation and/or Idaho Technology, Inc., to use only the enclosed amount of product according to the specified protocols. No right is conveyed, expressly, by implication, or by estoppel, to use any instrument or system under any claim of such U.S. patent(s), other than for the amount of product contained herein.

Literature Code: AB-4323-v7-0411