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Thermo Scientific ABsolute Fast QPCR Low ROX Mix

Description

ABsolute™ Fast QPCR 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-Fast™ 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 5 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 Fast QPCR Low ROX Mix after aliquoting into the reaction well.
- dNTPs, including dTTP to improve reaction sensitivity and efficiency compared to dUTP.
- ROX, passive reference dye for normalization of data.

Kit Contents

Vial	Pack Size (cap color)	
	A	B
ABsolute Fast QPCR Low ROX Mix (2X)	5ml (clear)	10 x 5ml (clear)

Cycler & Probe Compatibility

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

* **For RNA templates**, we recommend our Verso™ cDNA Synthesis Kit (AB-1453/B) for the reverse transcription step.

INFORMATION

Thermo-Fast™ DNA Polymerase

The enzyme requires an activation step at 95°C for 5 minutes.

Thermo-Fast™ 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.

Storage Conditions

Store at -20°C until ready for use. ABsolute™ Fast QPCR Low ROX Mix is stable for a minimum of 12 months. The reagents can be stored at 4°C for up to 3 months. Avoid repeated freeze thawing. The ROX dye is 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.
- The recommended amplicon length is in the range of 60 to 300 bp although optimal results may be obtained with amplicons <150bp.
- As best performance is achieved with dTTP, the ABsolute Fast QPCR Low ROX Mix contains a nucleotide mix with dTTP instead of dUTP.

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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 Fast QPCR 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	ABsolute Fast QPCR Low ROX Mix (2X)	12.5 µl	1X
	Forward primer (10 µM) [a]	1 µl	400 nM
	Reverse primer (10 µM) [a]	1 µl	400 nM
	Probe	Variable	100 - 250 nM
	Water (PCR grade) [b]	Variable	
	Template (DNA or cDNA) [c]	1 - 5 µl	<250 ng/reaction
	Total volume	25 µl	

Example of a **fast QPCR thermal cycling program**:

	Temp.	Time	Number of cycle
Enzyme activation	95°C	**5 min**	1 cycle
Denaturation	95°C	1 sec [d]	40 cycles
Annealing/Extension	60°C	20 sec [d]	

Notes

a – Start by using 400 nM of each primer. If optimization is required, try stepping the primer concentration up or down in 25nM increment between 100 nM and 500 nM final concentration.

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. Routinely, only 1 µl of template DNA or cDNA should be added to reduce carryover of PCR inhibitors. This volume can be increased up to 5 µl for low copy number templates.

d – **The fast thermal protocol shown may require optimization for best results. Some assays may require longer dwell times for efficient amplification. If so, increasing denaturation and annealing/extension dwell times in 5 second increment may be beneficial. Conversely, the dwell times may be decreased for some amplicons offering even faster QPCR results. Please note that it may be necessary to redesign the assay with a shorter amplicon (<150bp) to support fast thermal cycling.**

Quality control

ABsolute Fast QPCR Low ROX Mix is tested functionally using QPCR. The product must demonstrate linearity of amplification over a specified serial dilution of human genomic DNA under fast cycling conditions.

Ordering Information

AB-4329/A	ABsolute™ Fast QPCR Low ROX Mix	200 x 25 µl rxns
AB-4329/B	ABsolute™ Fast QPCR Low ROX Mix	1,600 x 25 µl rxns
AB-4330/A	ABsolute™ Fast QPCR Low ROX Mix	400 x 25 µl rxns
AB-4330/B	ABsolute™ Fast QPCR Low ROX Mix	4,000 x 25 µl rxns

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

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