

Absolute™ Fast QPCR Mix Plus ROX Vial

Description

Absolute™ Fast QPCR Mix Plus ROX Vial 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 fast, sensitive and reproducible QPCR reaction:

- Thermo-Fast™ 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 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 MgCl₂ 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 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 (separate vial).

Kit Contents

Vial	Pack Size (cap color)	
	A	B
Absolute Fast QPCR Mix (2X)	2 x 1.25ml (clear)	16 x 1.25ml (clear)
ROX Reference Dye (1 mM)	25µl (brown)	25µl (brown)

Cycler & Probe Compatibility

Absolute™ Fast QPCR Mix Plus ROX Vial is compatible for use with any probe system and with all block-based QPCR instruments and the Rotor-Gene™.

* 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. A separate vial of ROX is included in this kit for optional addition to the ABsolute Fast QPCR Mix. The final concentration will vary depending on the real time cycler. For example, for a concentration of 100 nM ROX in a final 1X QPCR reaction mix, dilute ROX (1 mM) 40 times i.e. 5 µl ROX Reference Dye + 195 µl PCR grade water and add 10 µl of the diluted ROX solution to each 1.25 ml vial of ABsolute Fast QPCR Mix or 40 µl to each 5 ml vial of ABsolute Fast QPCR Mix.

Storage Conditions

Store at -20°C until ready for use. ABsolute™ Fast QPCR Mix Plus ROX Vial 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 Mix contains a nucleotide mix with dTTP instead of dUTP.

DIRECTIONS FOR USE

Tips and Protocol

Thaw the reagents on ice, mix the solutions and spin down before use to recover the maximum amount. **Do not vortex the Absolute Fast QPCR 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 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 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-4325/A	ABsolute™ Fast QPCR Mix Plus ROX Vial	200 x 25 µl rxns
AB-4325/B	ABsolute™ Fast QPCR Mix Plus ROX Vial	1,600 x 25 µl rxns
AB-4326/A	ABsolute™ Fast QPCR Mix Plus ROX Vial	400 x 25 µl rxns
AB-4326/B	ABsolute™ Fast QPCR Mix Plus ROX Vial	4,000 x 25 µl rxns

Related Products

Cat. No.	Description	Quantity
AB-0600/W	Thermo-Fast™ 96 Non-Skirted, white *	25 plates
AB-0800/W	Thermo-Fast™ 96 Skirted PCR Plate, white *	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

Troubleshooting

For troubleshooting, see www.abgene.com/troubleshoot.asp or contact Thermo Fisher Scientific (ABgene) TechSupport at abgene.techsupport@thermofisher.com

UK TechSupport, call +44 (0) 1372 840 410

For all other regions, please contact your local Thermo Fisher Scientific (ABgene) office / distributor.

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