

## Thermo Scientific ABsolute QPCR ROX Mix

### Description

ABsolute™ QPCR 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 *Taq* 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.
- ROX, passive reference dye for normalization of data.

### Kit Contents

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

### Cycler & Probe Compatibility

ABsolute™ QPCR ROX Mix is compatible for use with any probe system and qPCR cyclers requiring high ROX dye level, including ABI PRISM® 7000, 7300, 7700, 7900 and 7900HT (including Fast-Block).

\* For RNA template, use Verso 1-Step QRT-PCR ROX Kit (AB-4101)

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).

**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 500 nM.

**Storage Conditions**

Store at -20°C until ready for use. ABsolute™ QPCR 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 dye is light sensitive; exposure should be minimized. Shipped on ice within the UK and on dry ice internationally 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 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 QPCR 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
Absolute QPCR ROX Mix (2X)	12.5 µl	1X
Forward primer (10 µM) <sup>a</sup>	1 µl	400 nM
Reverse primer (10 µM) <sup>a</sup>	1 µl	400 nM
Probe	Variable	100 - 250 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/Extension <sup>d</sup>	60°C	60 sec	

**Notes**

- a – For optimization, a primer titration should be performed from 100 nM to 500 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 – Separate annealing (50–60°C for 30 sec) and extension steps (72°C for 30 sec) may be necessary with some probe systems (e.g. Molecular Beacons), as the optimal temperature for detecting fluorescence may be different.

### Quality control

ABsolute QPCR 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-1138/A	ABsolute™ QPCR ROX Mix	200 x 25 µl rxns
AB-1138/B	ABsolute™ QPCR ROX Mix	1,600 x 25 µl rxns
AB-1139/A	ABsolute™ QPCR ROX Mix	400 x 25 µl rxns
AB-1139/B	ABsolute™ QPCR ROX Mix	4,000 x 25 µl rxns

### Related Products

Cat. No.	Description	Quantity
AB-4138/A	ABsolute™ Blue QPCR ROX Mix	2 x 1.25 ml
AB-1100/W	ABgene 96-Well PCR Plate (Raised Semi-Skirt, white) *	25 plates
AB-1400/W	ABgene 96-Well PCR Plate (Semi-Skirted, Flat-Deck, white) *	25 plates
AB-1170	ABsolute™ QPCR Adhesive Seal	50 sheets
AB-0866	Ultra Clear Cap Strips (8 caps)	120 strips

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

### Troubleshooting

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

	United States	Rest of World
<b>Email</b>	<a href="mailto:abgeneus.techservice@thermofisher.com">abgeneus.techservice@thermofisher.com</a>	<a href="mailto:abgene.techsupport@thermofisher.com">abgene.techsupport@thermofisher.com</a>
<b>Phone</b>	+1 (800) 235-9880	+44 (0)1372 840410

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