Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific ABsolute Blue qPCR Mix Plus ROX Vial

#AB-4136/B 16 x 1.25 mL

Lot _ **Expiry Date _**

Ordering Information

Component	#AB-4136/B 1600 rxns of 25 µL	#AB-4137/A 400 rxns of 25 µL
2X ABsolute Blue qPCR Mix	16 × 1.25 mL	5 mL
1 mM ROX Reference Dye	25 µL	25 µL

Store at -20°C

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Description

Thermo Scientific ABsolute Blue 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 rapid, sensitive and reproducible qPCR reaction:

- Thermo Scientific Thermo-Start DNA Polymerase, a chemically modified hot-start version of Thermo Scientific ThermoPrime Plus DNA Polymerase, which prevents non-specific amplification during the reaction set-up. Thermo-Start™ has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading). **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 qPCR Mix after aliquoting into the reaction well.
- dNTP's, including dTTP to improve reaction sensitivity and efficiency compared to dUTP.
- ROX, passive reference dye for normalization of data (separate vial).

Cycler Compatibility

ABsolute Blue qPCR Mix Plus ROX Vial is compatible for use with any probe system and with all block-based qPCR instruments and the Rotor-Gene™.

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 Blue 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 1 mM ROX 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 Blue qPCR Mix or 40 µL to each 5 mL vial of ABsolute Blue qPCR Mix.

Storage Conditions

Store at -20 °C until ready for use. ABsolute Blue qPCR Mix Plus ROX Vial 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.

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 Mix contains a nucleotide mix with dTTP instead of dUTP.

Protocol

Thaw the reagents on ice, mix the solutions and spin down before use to recover the maximum amount. **Do not vortex the ABsolute Blue 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
2X ABsolute Blue qPCR Mix	12.5 µL	1X
Forward primer (1 µM)*	1 µL	400 nM
Reverse primer (1 µM)*	1 µL	400 nM
Probe	variable	100–250 nM
Template (DNA or cDNA)**	1-5 µL	< 250 ng/rxn
Water, nuclease-free (#R0581)	To 25 µL	
Total volume	25 µL	

*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.

**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.

Example of qPCR thermal cycling program:

	Temp.	Time	Number of cycles
Enzyme activation	95 °C	15 min	1 cycle
Denaturation	95 °C	15 s	40 cycles
Annealing/Extension *	60 °C	60 s	

* Separate annealing (50–60 °C for 30 s) and extension steps (72 °C for 30 s) may be necessary with some probe systems (e.g. Molecular Beacons), as the optimal temperature for detecting fluorescence may be different.

CERTIFICATE OF ANALYSIS

ABsolute Blue qPCR Mix is tested functionally using qPCR. The product must demonstrate linearity of amplification over a specified serial dilution of human genomic DNA.

Quality authorized by:  Jurgita Zilinskiene

NOTICE TO PURCHASER

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 6,127,155, 5,677,152 (claims 1 to 23 only) and 5,773,258 (claims 1 and 6 only). Use of this product in a passive reference method is covered by the following U.S. Patent: 5,928,907 (claim numbers 12-24, 27-28) and corresponding patent claims outside the US. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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