Thermo
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PRODUCT INFORMATION

Thermo Scientific ABsolute qPCR Mix

#AB-1133/A 5 mL

Lot _ **Expiry Date _**

Ordering Information

Component	#AB-1133/A 400 rxns of 25 µL	#AB-1132/B 1600 rxns of 25 µL
Absolute qPCR Mix	1 × 5 mL	16 × 1.25 mL

Store at -20°C

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Description

Thermo Scientific ABsolute qPCR 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 Scientific Thermo-Start DNA Polymerase, a chemically modified hot-start version of Thermo Scientific ThermoPrime DNA Polymerase, which prevents non-specific amplification during the reaction set-up. **This 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).
- 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.
- dNTP's, including dTTP to improve reaction sensitivity and efficiency compared to dUTP.

Cycler & Probe Compatibility

ABsolute™ qPCR Mix is compatible for use with any probe system and with all qPCR machines that do not require reference dye.

Storage Conditions

Store at -20°C until ready for use. The reagents can be stored at 4°C for up to 1 month. Avoid repeated freeze thawing.

Additional Info

The use of disposable gloves, RNase and DNase 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 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 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
ABsolute QPCR Mix (2X)	12.5 µL	1X
Forward primer (10 µM)*	1 µL	400 nM
Reverse primer (10 µM)*	1 µL	400 nM
Probe		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 protocol:

	Temp.	Time	Number of cycles
Enzyme activation	95°C	15 min	1 cycle
Denaturation	95°C	15 s	40 cycles
Anealing/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 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

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.thermoscientific.com/onebio for Material Safety Data Sheet of the product.