Thermo SCIENTIFIC

**PRODUCT INFORMATION**

# Thermo Scientific ABsolute qPCR Low ROX Mix

**#AB-1318/B** 16 x 1.25 mL

**Lot _** **Expiry Date _**

## Ordering Information

| Component | #AB-1318/B 1600 rxns of 25 µL | #AB-1319/A 400 rxns of 25 µL |
|---|---|---|
| ABsolute qPCR Low ROX Mix | 16 × 1.25 mL | 5 mL |

**Store at -20°C**

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## Description

Thermo Scientific ABsolute 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 Scientific Thermo-Start DNA Polymerase, a chemically modified hot-start version of Thermo Scientific 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.** 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.
- ROX, passive reference dye for normalization of data. The concentration of ROX in the final 1X reaction is 25 nM.

## Cycler & Probe Compatibility

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

## 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. The ROX dye is light sensitive; exposure should be minimized.

## 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 Low ROX 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 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 |
|---|---|---|
| **ABsolute qPCR Low ROX 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 Low ROX 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.

**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.