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# Thermo Scientific ABsolute QPCR Low ROX Mix

## Description

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-Start™ 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 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.
- 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 Low ROX Mix (2X) | 5ml (clear) | 10 x 5ml (clear) |

## Cycler & Probe Compatibility

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

* **For RNA template**, use Verso 1-Step QRT-PCR Low ROX Kit (AB-4102)

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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 25 nM.

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## Storage Conditions

Store at -20°C until ready for use. ABsolute™ QPCR Low 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 for international and within the US.

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## 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 Low ROX Mix contains a nucleotide mix with dTTP instead of dUTP.

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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 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 |
|---|---|---|---|
| Reaction Mix | ABsolute QPCR Low ROX 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 **QPCR thermal cycling program**:

| | Temp. | Time | Number of cycle |
|---|---|---|---|
| Enzyme activation | 95°C | **15 min** | 1 cycle |
| Denaturation | 95°C | 15 sec | 40 cycles |
| Annealing/Extension [d] | 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 the carryover of any PCR inhibitor. 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.

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## Quality control

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.

## Ordering Information

| | | |
|---|---|---|
| AB-1318/A | ABsolute™ QPCR Low ROX Mix | 200 x 25 µl rxns |
| AB-1318/B | ABsolute™ QPCR Low ROX Mix | 1,600 x 25 µl rxns |
| AB-1319/A | ABsolute™ QPCR Low ROX Mix | 400 x 25 µl rxns |
| AB-1319/B | ABsolute™ QPCR Low ROX Mix | 4,000 x 25 µl rxns |

## Related Products

| Cat. No. | Description | Quantity |
|---|---|---|
| AB-4318/A | ABsolute™ Blue QPCR Low ROX Mix | 2 x 1.25 ml |
| AB-0600/W | Thermo-Fast™ 96 Non-Skirted, white * | 25 plates |
| AB-1100/W | Thermo-Fast™ 96 PCR Detection Plate, white * | 25 plates |
| AB-1400/W | Thermo-Fast™ 96 PCR Detection Plate Mark II, 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

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

| **Troubleshooting:** | **Email** | **Phone** |
|---|---|---|
| North America (US, Canada, Central/South America) | Techservice.genomics @thermofisher.com | +1 (800) 235-9880 |
| Europe (EU, Middle East, Africa) | Techservice.emea.genomics @thermofisher.com | (+) 44 1372 840410 |
| Other Countries | www.thermoscientific.com/ dharmacondistributors | |

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