For general laboratory use.

Print

EagleTaq Universal Master Mix (ROX)

2× concentrated, ready-to-use hot start master mix for qPCR and qRT-PCR using the hydrolysis probe detection format on real-time PCR instruments

Cat. No. 07 260 288 190 1 ml
Cat. No. 07 260 296 190 5 ml
Cat. No. 07 249 926 190 10 × 5 ml

Version 02
Content version: October 2014

Store the kit at −15 to −25°C

1. What this Product Does

Number of Reactions

- 100 reactions of 20 µl (1 ml pack size)
- 500 reactions of 20 µl (5 ml pack size)
- 5,000 reactions of 20 µl (10 × 5 ml pack size)

Contents

Vial	Contents
EagleTaq Universal Master Mix (ROX), 2× conc.	1 × 1 ml (07 260 288 190) 1 × 5 ml (07 260 296 190) 10 × 5 ml (07 249 926 190) • Contains EagleTaq DNA Polymerase and a reaction buffer with dATP, dCTP, dGTP, dUTP, $MgCl_2$, and ROX.

Storage and Stability

The unopened kit is stable at −15 to −25°C through the expiration date printed on the label.

Once opened, EagleTaq Universal Master Mix (ROX) may be stored at +2 to +8°C for up to 3 months.

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

Additional Equipment and Reagents Required

Additional equipment and reagents required to perform real-time PCR assays with EagleTaq Universal Master Mix (ROX) include:

- standard laboratory equipment
  - pipettes with nuclease-free, aerosol-resistant pipette tips
  - sterile reaction tubes for preparing PCR mixes and dilutions
  - standard benchtop microcentrifuge
- for first-strand cDNA synthesis (optional, for RNA target amplification only)
  - Transcriptor First Strand cDNA Synthesis Kit*
- for real-time PCR
  - real-time PCR instrument
  - PCR reaction vessels (*e.g.,* optical tubes or microplates)
  - sequence-specific primers and probes
  - Water, PCR Grade*
- for carryover prevention (optional)
  - Uracil-DNA Glycosylase (UNG)*

Application

- The EagleTaq Universal Master Mix (ROX) is a ready-to-use, 2× concentrated PCR master mix that contains all the reagents (except primers, probes, and template) needed for performing quantitative, real-time PCR hydrolysis probe reactions. It contains a special ROX reference dye which makes it suitable for all real-time instruments on which a ROX reference dye is needed for quantitative analysis.
- The EagleTaq Universal Master Mix (ROX) contains dUTP so that it may be used with Uracil-DNA Glycosylase (UNG) to prevent false positives arising from carryover contamination.
- The hot start properties allow reaction setup at ambient temperature.
- With this robust reagent, any PCR protocol optimization is minimized.

2. How to Use this Product

2.1 Before You Begin

General Considerations

The EagleTaq Universal Master (ROX) can be used for the amplification and detection of any DNA or cDNA target. However, you need to adapt your detection protocol to the reaction conditions of the particular real-time PCR instrument in use and design a specific hydrolysis probe and PCR primers for each target. See the Operator's Manual of your real-time PCR instrument for general recommendations.

Primers

Use PCR primers at a final concentration of 0.2 - 1 µM each. A recommended starting concentration is 0.5 µM each.

The EagleTaq Universal Master Mix (ROX) is designed for optimal amplification of targets up to 500 bp long. The master mix is not optimized for long templates.

Probe

The probe concentration should be lower than the primer concentration. As a starting point, we recommend using 0.2 µM probe. However, suitable concentrations range from 0.1 µM to 0.2 µM.

Sample Material

Use any template DNA (*e.g.*, genomic or plasmid DNA, cDNA) suitable for PCR in terms of purity, concentration, and absence of inhibitors. For reproducible isolation of nucleic acids, we recommend using:

- the MagNA Pure 96 Instrument*, the MagNA Pure LC Instrument*, or the MagNA Pure Compact Instrument* together with a dedicated nucleic acid isolation kit (for automated isolation), or
- a High Pure Nucleic Acid Isolation Kit* (for manual isolation)

Use 10 pg to 500 ng complex genomic DNA or 0.1 to 10 ng plasmid DNA/cDNA. Recommended starting concentrations are up to 250 ng genomic DNA or 50 ng cDNA.

Dilute the template DNA either in Water, PCR Grade* or 5 to 10 mM Tris-HCl (pH 7 to 8).

2.2 Procedure

Step	Action
❶	Thaw primer, probe, and nucleic acid template solutions, and mix by vortexing.
❷	Prepare PCR primer and probe solutions (*e.g.,* in a concentration of 10 µM for each primer, and of 4 µM for the probe).
❸	Vortex the EagleTaq Universal Master Mix (ROX).
❹	Spin down all vials in a microcentrifuge prior to opening to ensure recovery of the whole volume.
❺	To a sterile reaction tube, add the components in the order listed below (*e.g.,* for each 20 µl reaction):

Reagent	Vol.	Final Conc.
EagleTaq Universal Master Mix (ROX), 2× conc.	10 µl	1× conc.
Forward primer, 10 µM	1 µl	0.5 µM
Reverse primer, 10 µM	1 µl	0.5 µM
Hydrolysis Probe, 4 µM	1 µl	0.2 µM
Water, PCR Grade	2 µl	
Final Volume	**15 µl**	

To prepare the PCR mix for more than one reaction, multiply the amounts in the "Volume" column by z, where z = the number of reactions to be run + one additional reaction.

❻	Mix by pipetting.
❼	In case of multiple reactions, dispense 15 µl of the reaction mix into individual PCR reaction tubes or wells of a multiwell plate.
❽	Add 5 µl nucleic acid template.
❾	Mix by pipetting, seal the tubes or plate, and centrifuge briefly.
❿	Place the samples into a real-time PCR instrument.
⓫	Follow the Operator´s Manual of your thermal cycler supplier to program the instrument with the following parameters:

Step	Cycles	Time	Temp
UNG (optional)	1	2 min	52°C
Activation	1	10 min	95°C
PCR Amplification	45		
• Denaturation		• 15 sec	• 95°C
• Annealing		• 60 sec	• 60°C
• Elongation (optional)		• 1 sec	• 72°C
Cooling	1	30 sec	40°C

EagleTaq Universal Master Mix (ROX) contains dUTP, but does not contain UNG enzyme. If UNG carryover contamination protection is desired, add UNG according to the respective Instruction for Use.

⓬	Refer to your instrument user guide for instructions to start the reaction.
⓭	At the end of the reaction, follow instrument instructions for quantification/analysis.

2.3 Optimization

In case the recommended protocol does not fulfill the assay requirements, the reaction might be optimized by increasing the annealing/elongation temperature to +63°C for higher specificity or use longer annealing/elongation holding times in case of longer PCR products.

3. Troubleshooting

Problem	Possible Cause	Recommendation
No amplification/no product detectable	Error in the PCR program	Adjust the PCR program.
	Pipetting errors (*e.g.,* nucleic acid template not added)	Repeat experiment; check pipetting steps carefully.
	Amplicon too long (> 500 bp)	• Redesign primers to shorten the PCR product. • Prolong annealing/elongation time.
	Suboptimal primer design	Redesign primers.
	Inhibitory effects by impurities of the nucleic acid template	Repeat the isolation of the nucleic acid template.
	Incorrect filter settings	Confirm that data are collected with correct filter combinations.
Fluorescence varies within a run	Instrument not correctly calibrated	Recalibrate the instrument.
	Probe variations	Keep dye-labeled reagents such as probes away from light.
Amplification products in the negative (no template) control	Contamination with nucleic acid templates	• Replace solutions in which a contamination might occur (*e.g.,* water). • Re-run with fresh reagents. • Clean lab environment (*e.g.,* bench). • Use UNG to prevent carryover contamination.

4. Additional Information on this Product

How this Product Works

The EagleTaq Universal Master Mix (ROX), 2× concentrated, contains all reagents (except primers, probe, and nucleic acid template) needed for polymerase chain reaction assays. The EagleTaq Universal Master Mix (ROX) contains EagleTaq DNA Polymerase for hot start PCR to improve specificity and sensitivity of the PCR by minimizing the formation of nonspecific amplification products. EagleTaq DNA Polymerase is chemically modified and requires a high temperature hold for activation.
As the EagleTaq Universal Master Mix (ROX) contains ROX, it allows the detection of the released signal in relationship to the reference dye ROX.

Prevention of Carryover Contamination

Uracil-DNA Glycosylase (UNG) is suitable for preventing carryover contamination in PCR. This cross-contamination prevention technique involves incorporating deoxyuridine triphosphate into amplification products, permitting pretreatment of subsequent PCR mixtures with UNG. When a dUTP containing contaminant is present in later PCRs, it will be cleaved by a combination of UNG and the high temperatures of the initial denaturation step; it will not serve as a PCR template. Since target DNA templates contain deoxythymidine rather than deoxyuridine, it is not affected by this procedure.

Quality Control

Each lot of the EagleTaq Universal Master Mix (ROX) is function tested in PCR using a control template DNA plasmid, primers and a FAM-labeled hydrolysis probe specific for the ITGA4 gene.

5. Supplementary Information

5.1 Conventions

Text Conventions

To make information consistent and memorable, the following text conventions are used in this Instructions for Use:

Text Convention	Use
Numbered instructions labeled ❶, ❷, etc.	Steps in a procedure that must be performed in the order listed.
Numbered instructions labeled ①, ②, etc.	Steps in a process that usually occur in the order listed.
Asterisk *	Denotes a product available from Roche.

Symbols

In this document, the following symbols are used to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.

5.2 Changes to Previous Version

- Update regarding UNG concentration
- Editorial changes

5.3 Disclaimer of License

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5.4 Trademarks

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5.5 Regulatory Disclaimer

For general laboratory use.

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To call, write, fax, or email us, visit **www.lifescience.roche.com**, and select your home country. Country-specific contact information will be displayed.

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