For general laboratory use.

EagleTaq Universal Master Mix (ROX)

Version: 04

Content version: October 2018

2x 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. 07 249 926 190	10 x 5 ml 5,000 reactions of 20 µl final volume each 2x conc.
Cat. No. 07 260 288 190	1 ml 100 reactions of 20 µl final volume each 2x conc.
Cat. No. 07 260 296 190	5 ml 500 reactions of 20 µl final volume each 2x conc.

Store at −15 to −25°C

1. General Information

1.1. Contents

Label	Function / Description	Catalog Number	Content
EagleTaq Universal Master Mix (ROX), 2x conc.	Contains EagleTaq DNA Polymerase and a reaction buffer with dATP, dCTP, dGTP, dUTP, $MgCl_2$, and ROX.	07 260 288 190	1 vial, 1 ml
		07 260 296 190	1 vial, 5 ml
		07 249 926 190	10 vials, 5 ml each

1.2. Storage and Stability

Storage Conditions (Product)

The unopened mix 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 to light should be minimized.

1.3. 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)*

1.4. Application

- The EagleTaq Universal Master Mix (ROX) is a ready-to-use, 2x 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

Sample Materials

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:

- Either a MagNA Pure System 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).

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.

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.

2.2. Protocols

Follow the procedure below to prepare one 20µl standard reaction.

1. Thaw primer, probe and nucleic acid template solutions, mix by vortexing.

2. Prepare PCR primer and probe solutions (*e.g.*, in a concentration of 10 µM for each primer, and of 4 µM for the probe).

3. Vortex the EagleTaq Universal Master Mix (ROX).

4. Spin down all vials in a microcentrifuge prior to opening to ensure recovery of the whole volume.

5. To a sterile reaction tube add the components in the order listed below (for each 20 µl reaction):

Reagent	Volume	Final conc.
EagleTaq Universal Master Mix (ROX), 2x conc.	10 µl	1x 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 PCR reaction mixes for more than one reaction, multiple the amount in the column "Volume" by the number of reactions plus sufficient additional reactions.

6. Mix by pipetting.

7. In case of multiple reactions, dispense 15 µl of the reaction mix into individual PCR reaction tubes or wells of a multiwell plate.

8. Add 5 µl nucleic acid template.

9. Mix by pipetting, seal the tubes or plate, and centrifuge briefly.

10. Place the samples into a real-time PCR instrument.

11 Follow the Operator´s Manual of your thermal cycler supplier to program the instrument with the following parameters:

Step	Cycles	Time	Temperature
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.

12 Refer to your instrument user guide for instructions to start the reaction.

13 At the end of the reaction, follow instrument instructions for quantification/analysis.

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.

2.3. Other Parameters

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.

3. Troubleshooting

Observation	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

4.1. Test Principle

The EagleTaq Universal Master Mix (ROX), 2x 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.
EagleTaq Universal Master Mix (ROX) is a 2x concentrated, ready-to-use hot start master mix for qPCR and qRT-PCR using the hydrolysis probe detection format on real-time PCR instruments.

4.2. 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

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

5.2. Changes to previous version

Editorial changes.

5.3. Trademarks

EAGLETAQ, HIGH PURE and MAGNA PURE are trademarks of Roche.
All third party product names and trademarks are the property of their respective owners.

5.4. License Disclaimer

For patent license limitations for individual products please refer to: **List of LifeScience products**

5.5. Regulatory Disclaimer

For general laboratory use.

5.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

5.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

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

1018.07265565001 ©

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