

# EcoNova HotTaq EvaGreen® qPCR Premix

With ROX dye

Reference: AB13009; AB13010; AB13011



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## INTENDED USE AND PRESENTATION:

This HotTaq EvaGreen® qPCR Premix is widely used in molecular biology research.

**AB13009, 1 mL.** 1 mL.

**AB13010, 3 mL.** 1 mL x 3.

**AB13011, 5 mL.** 1 mL x 5.

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTPs into acid-insoluble material in 30 minutes at 74°C.

For research use only.

## SUMMARY, EXPLANATION AND LIMITATIONS:

EcoNova HotTaq EvaGreen® (registered by Biotium, Inc., Hayward, CA, USA) Probe qPCR Mix (ROX) is optimized for real-time quantitative PCR assays. The ready-to-load mix includes EcoNova HotTaq DNA polymerase, ultrapure dNTPs, MgCl<sub>2</sub>, EvaGreen® dye and ROX dye according to system requirements. Only water, template and primers needs to be added.

EcoNova HotTaq DNA polymerase is activated by a 15 min incubation step at 95°C. This prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during qPCR setup.

**EvaGreen® Dye:** EvaGreen® is a DNA-binding dye for qPCR that, compared to SYBR® Green I (registered by Molecular Probes, Inc.), has similar spectra but much less PCR inhibition. It is extremely stable and has been shown to be nonmutagenic and noncytotoxic.

EvaGreen® is compatible with all common real-time PCR cyclers – simply select the standard settings for SYBR® Green or FAM.

## APPLICATIONS:

EcoNova HotTaq qPCR Premix for Probe with ROX is a premix for all your everyday qPCR reactions with detection and quantification of DNA and cDNA targets, profiling gene expression, High Resolution Melt (HRM) Analysis, microbial detection and viral load determination.

## PRODUCT COMPOSITION:

-EcoNova HotTaq DNA Polymerase

-5X qPCR buffer

-12,5 mM MgCl<sub>2</sub>

-dNTPs, including dTTP to improve reaction sensitivity and efficiency compared to dUTP.

- EvaGreen® dye

-ROX dye, an internal passive reference dye used to normalize the fluorescent reported signal generated in qPCR.

## METHODS AND PROCEDURE:

Optimal reaction conditions, such as reaction time, temperature, and amount of template DNA, may vary and must be individually determined.

### General Reaction Protocol:

1. Thaw 5X EcoNova HotTaq qPCR Premix.

2. Prepare a master mix.

## Recommended qPCR reaction mix:

Component	Volume	Final Conc.
5X EcoNova HotTaq EvaGreen® qPCR Premix	4 µL	1X
Upstream Primer (10 pmoles/µL)	0,16-0,5 µL	80-250 nM
Downstream Primer (10 pmoles/µL)	0,16-0,5 µL	80-250 nM
Template DNA	1-5 µL	1-50 ng/µL
Sterilized D.W.	Up to 20 µL	-
Total Volume	20 µL	-

\*Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings (<http://frodo.wi.mit.edu/primer3/>).

3. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes. Centrifuge the reactions in a microcentrifuge for 10 seconds.

4. Perform PCR using your standard parameters (3-step cycling).

Step	Temp.	Time	Cycles
Initial denaturation	95°C	15 min	1
Denaturation	95°C	15 sec.	40
Annealing	60-65°C	20 sec.	
Final extension	72°C	20 sec.	

\***IMPORTANT:** To activate the polymerase, include an incubation step at 95°C for 12 - 15 minutes at the beginning of the PCR cycle.

## REQUIRED MATERIALS BUT NOT SUPPLIED:

All reagents, materials, and laboratory equipment for qPCR procedures are not provided with this premix. This includes sterile reaction tubes, micropipettes and tips, template DNA, gen-specific PCR primer pair, dNTPs mixture, PCR grade H<sub>2</sub>O, heat pretreatment equipment (thermoblock, microwave), centrifuge, cold store and thermal block cycler.

Buffered solutions for DNA extraction or purification, enzyme treatments, highly sensitive detection systems, and other auxiliary reagents are available from Gennova Scientific.

## STORAGE AND STABILITY:

Shipping and temporary storage for up to 1 month at room temperature or storage for up to 6 months at 2-8°C has no detrimental effects on the quality of EcoNova HotTaq Premix. Store at -20°C until the expiration date printed on product label. Avoid prolonged exposure to light. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. Do not use after the expiration date. If the product is stored under different conditions from those stipulated in these technical indications, the new conditions must be verified by the user. The validity period of the ready to use products when opened, is the same as the expiration date indicated on the label of intact product.

Gennova Scientific guarantees that the product will maintain all of the described characteristics from the production date until the expiration date, as long as the product is stored and used as recommended. No other guarantees are provided. Under no circumstances Gennova Scientific is obliged to cover damages caused by use of this reagent.



Catalog number



Batch code



Research use only



Temperature limitation



Expiration date



Manufacturer



See instruction for use



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### TROUBLESHOOTING:

If unusual amplification is observed or any other deviations from the expected results, please read these instructions carefully, along with the instructions from the PCR system. If this does not solve the problem, please contact Genova Scientific's technical support department ([techsupport@genovalab.com](mailto:techsupport@genovalab.com)) or your local distributor.

### PRECAUTIONS:

Use only by qualified personnel.

Use proper protective equipment in order to avoid contact with reagents and samples in the eyes, skin, and mucosal tissues. In case of contact with sensitive areas, immediately flush the affected area with water. Avoid microbial contamination of the reagent, as this may produce nonspecific amplification results.

Material safety data sheet (MSDS) is available upon request.

### PERFORMANCE CHARACTERISTICS:

Genova Scientific has performed studies to evaluate the functioning of this polymerase for use with standard amplification systems, concluding that the product is both specific and sensitive for PCR performance.

### BIBLIOGRAPHY:

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