TOOLS 5X EvaGreen qPCR Mix with ROX

Cat. no. TF-5EVR250

Storage: −20°C for up to 1 year

Product Size: 1 mL

Introduction

The TOOLS 5× EvaGreen qPCR Mix with ROX is optimized for real-time quantitative polymerase chain reaction (PCR) assays. The ready-to-load mix includes TOOLS HotTaq DNA polymerase, ultrapure dNTPs, $MgCl_2$, EvaGreen® dye, and ROX dye according to system requirements. Only water, template, and primers need to be added. TOOLS HotTaq DNA polymerase is activated by a 15-min incubation step at 95°C. This prevents the extension of nonspecifically annealed primers and primer-dimers formed at low temperatures during qPCR.

EvaGreen® Dye: EvaGreen® is a DNA-binding dye for qPCR that, compared with SYBR® Green I, has similar spectra but much lower 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

ROX Dye: ROX is an internal passive reference dye used to normalize the fluorescent reporter signal generated in qPCR.

Composition

TOOLS HotTaq DNA polymerase

5× qPCR buffer E

12.5 mM $MgCl_2$ (1× PCR solution–2.5 mM $MgCl_2$)

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

EvaGreen® dye

ROX dye

Cycler compatibility

The TOOLS 5× EvaGreen qPCR Mix with ROX can be used with all major qPCR cyclers requiring a high ROX dye level, including ABI PRISM® 5700, 7000, 7300, 7700, 7900, and 7900HT (including Fast-Block) as well as Stratagene Mx3000P™, Mx3005P™, and Mx4000®.

Recommended qPCR reaction mix

Component	Volume	Concentration
TOOLS 5X EvaGreen qPCR Mix	4 µL	1x
Primer Forward (10 pmol/µl)	0.16–0.5 µL	80-250 nM
Primer Reverse (10 pmol/µl)	0.16–0.5 µL	80-250 nM
DNA template	1–5 µL	1–50 ng/µL
H_2O PCR grade	up to 20 µL	
Total	20 µL	

Recommended qPCR cycles

Cycle step	Temp.	Time	Cycles
Initial denaturation	95ºC	15 min	1
Denaturation	95ºC	15 s	40
Annealing	60º-65°C	20 s	
Elongation	72ºC	20 s	

Important: To activate the polymerase, incubate it at 95°C for 15 min at the beginning of the qPCR cycle.

The product is for research only and not for diagnostic and clinical use.