CAPITAL™ qPCR Green Mix, 4×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0501701 BR0501702	200 rxn of 20 µl 1000 rxn of 20 µl	1 ml CAPITAL qPCR Green Mix 5 × 1 ml CAPITAL qPCR Green Mix
BR0501801 BR0501802	200 rxn of 20 µl 1000 rxn of 20 µl	1 ml CAPITAL qPCR Green Mix LRox 5 × 1 ml CAPITAL qPCR Green Mix LRox
BR0501901 BR0501902	200 rxn of 20 µl 1000 rxn of 20 µl	1 ml CAPITAL qPCR Green Mix HRox 5 × 1 ml CAPITAL qPCR Green Mix HRox

COMPONENT	COMPOSITION
CAPITAL qPCR Green Mix	Optimized 4× qPCR Master Mix containing proprietary Green dye
LRox Mix / HRox Mix	Rox incorporated in the mix in low / high concentration
STORAGE	−20°C (until expiry date – see product label) Protect from light. Avoid multiple freeze thaw cycles by preparing aliquots.

FEATURES

- Best in-class performance in a wide range of applications
- Highly specific amplification and excellent signal to noise ratio
- High sensitivity in amplification of low-abundance DNA targets with a wide range of linearity

APPLICATIONS

- Standard and fast cycling qPCR with rapid extension rate for early Ct values
- Accurate and robust gene expression analysis
- Excellent performance in copy number variation analysis

DESCRIPTION

biotechrabbit™ CAPITAL qPCR Green Mix allows sensitive and specific amplification with an excellent signal to noise ratio and rapid extension rates. Extremely low-copy-number targets can be detected with high efficiency over several logs of template concentration, while primer-dimer formation is efficiently minimized.
CAPITAL qPCR Green Mix shows accurate and robust performance in wide range of applications, including gene expression and copy number variation analysis.
To enable the use of the kit on qPCR platforms with different reference dye concentration requirements, three kit formats are available: a one-step kit containing no ROX, as well as LRox and HRox versions containing ROX in the corresponding concentrations.

Info: Recommended annealing temperature is 2°C above primer Tm (use gradient PCR to optimize the annealing temperature).

ROX REFERENCE DYE

- See PCR cycler instruction for recommended concentration of ROX passive reference dye

PROTOCOL

Notes

- For efficient amplification under fast cycling conditions use amplicon lengths between 80 bp and 200 bp.
- The shorter the amplicon length the faster the reaction can be cycled. Use maximum 400 bp amplicons.
- Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings (http://frodo.wi.mit.edu/primer3/).

Prevention of PCR contamination

When assembling the amplification reactions, care should be taken to eliminate the possibility of contamination with undesired DNA.

- Use separate clean areas for preparation of samples and reaction mixtures and for cycling.
- Wear fresh gloves. Use sterile tubes and pipette tips with aerosol filters for PCR setup.
- Use only water and reagents that are free of DNA and nucleases.
- With every PCR setup, perform a contamination control reaction that does not include template DNA.

Basic Protocol

- Keep the master mix protected from light until you use it.
- Aliquot the master mix to minimize freeze-thaw cycles and light exposure.
- Thaw on ice and mix very well all reagents. Assemble and keep all reactions on ice.
- Use only high quality optically clear reaction plates and seals designed for fluorescence applications.
- Do not use corner wells or use a more robust seal.
- Reserve plate positions for positive (control DNA) and negative (water or buffer) controls.
- First pipette the primer mixture, then add the template and last the Master Mix.
- Before preparing mixes, calculate the volume needed according to the reaction number plus one extra.
- To have a better correlation, run the reactions in triplets.

COMPONENT	VOLUME	FINAL CONCENTRATION
Primer Mix (Reverse and Forward)	Variable	100–400 nM
Too high primer concentrations result in unspecific amplification and should be avoided.		
Template DNA	Variable	10 pg – 100 ng
Use diluted or undiluted cDNA from less than 1 µg RNA		
CAPITAL qPCR Green Mix, 4×	5 µl	1×
Nuclease free water	Variable	
Total volume	20 µl	

- Gently mix the reactions without creating bubbles (do not vortex). Bubbles will interfere with fluorescence detection.
- Place the reaction into the PCR cycler.

CYCLING PROGRAM

STEP	TEMPERATURE	TIME	CYCLES
Initial activation	95°C	2-3 min	1
Denaturation	95°C	10-15 s	40-45
Annealing/Extension*	(60-68°C)	30 s	

*Recommended annealing/extension temperature is primer Tm +2°C. Use gradient PCR to optimize the annealing temperature. Do not use temperatures below 60°C.
Do not exceed 30 seconds. For melt analysis refer to instrument instructions.

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Mix tested functionally in qPCR.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)/Sicherheitshinweise finden Sie in den SDS unter:
http://www.biotechrabbit.com/support/documentation.html.

USEFUL HINTS

- Visit Applications at www.biotechrabbit.com for more products and product selection guides.
- Most biotechrabbit products are available in custom formulations and bulk amounts.

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