

# qPCR Green Core Kit

Kit for real-time qPCR with green-fluorescent DNA stain

Real-Time PCR

Cat.-No.	Amount
PCR-333S	100 reactions x 50 µl
PCR-333L	500 reactions x 50 µl

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20 °C, avoid frequent thawing and freezing  
qPCR GreenBuffer must be stored in the dark

## Description

qPCR Green Core Kit is designed for the quantitative real-time analysis of DNA samples using the fluorescent DNA stain EvaGreen<sup>®</sup>. The fluorescent dye in the reaction buffer intercalates into the amplification product during the PCR process and enables the rapid analysis of target DNA without the need to synthesize sequence-specific labeled probes. The kit provides a powerful tool for quantification of sample DNA in a broad dynamic range of up to 6 orders of magnitude with exceptional sensitivity and precision.

The high specificity and sensitivity of the Core Kit is achieved by an optimized hot-start polymerase. Its activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of nonspecifically annealed primers and primer-dimer formation at low temperatures during PCR setup.

The included dNTP mix contains dUTP instead of dTTP and allows an UNG (Uracil-N-Glycosylase) treatment at the onset of thermal cycling to prevent carry-over contaminations of DNA from previous PCR reactions.

The reaction chemistry of the kit is optimized for block-based PCR instruments. The kit can also be used with ROX reference dye in PCR instruments that are compatible with the evaluation of the ROX signal. In this case, the ROX dye should be added as 1x concentration to the PCR reaction.

## qPCR Pol (red cap)

5 units/µl

## dNTP Mix incl. dUTP (white cap)

10 mM dATP, 10 mM dCTP, 10 mM dGTP,  
20 mM dUTP

## 10x qPCR GreenBuffer complete (green cap)

200 mM Tris-HCl (pH 8.5), KCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, EvaGreen,  
stabilizers, 30 mM MgCl<sub>2</sub>

## 10x qPCR GreenBuffer without MgCl<sub>2</sub> (blue cap)

200 mM Tris-HCl (pH 8.5), KCl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, EvaGreen,  
stabilizers

## MgCl<sub>2</sub> Stock Solution (yellow cap)

25 mM MgCl<sub>2</sub>

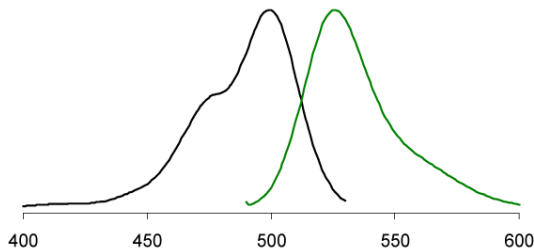
## EvaGreen<sup>®</sup> Fluorescent DNA Stain

EvaGreen<sup>®</sup> Fluorescent DNA Stain is a superior DNA intercalator dye specially developed for DNA analysis applications including real-time PCR (qPCR) and high-resolution DNA melting curve analysis (HRM). Upon binding to DNA, the non-fluorescent dye becomes highly fluorescent while showing no detectable inhibition to the PCR process. The dye is extremely stable both thermally and hydrolytically, providing convenience during routine handling.

The high quantum yield, excellent stability and lowest inhibition toward PCR makes it the ideal fluorophore in real-time PCR applications and a superior replacement for the widely used SYBR<sup>®</sup> Green I dye.

**Spectroscopic data**

 Excitation maximum:  $\lambda_{Ex} = 500$  nm (bound to DNA)

 Emission maximum:  $\lambda_{Em} = 530$  nm (bound to DNA)

 Excitation (left) and emission (right) spectra of EvaGreen<sup>®</sup> bound to dsDNA in PBS buffer (pH 7.3).

 To perform the EvaGreen-based assay simply select the optical setting for SYBR<sup>®</sup> Green or FAM on the detection instrument.

**Preparation of the qPCR master mix**

 The preparation of a master mix is crucial in quantitative PCR reactions to reduce pipetting errors. Prepare a master mix of all components except template as specified. A reaction volume of 20-50  $\mu$ l is recommended for most real-time instruments. Prepare 13 volumes of master mix for 12 samples or a triple-set of 4 samples. Pipet with sterile filter tips and minimize the exposure of the master mix, reaction buffer, and labeled DNA probe to light. Perform the setup in an area separate from DNA preparation or analysis. No-template controls should be included in all amplifications.

Component	Volume for 1x 50 $\mu$ l master mix	Volume for 13x 50 $\mu$ l master mix	Final conc.
10x qPCR Buffer complete (green cap)	5 $\mu$ l	65 $\mu$ l	1x conc.
dNTP Mix incl. dUTP (white cap)	1 $\mu$ l	13 $\mu$ l	200 $\mu$ M
Primer forward (10 $\mu$ M) <sup>1)</sup>	1.5 $\mu$ l	19.5 $\mu$ l	300 nM
Primer reverse (10 $\mu$ M) <sup>1)</sup>	1.5 $\mu$ l	19.5 $\mu$ l	300 nM
UNG (PCR-353) <sup>2)</sup>	0.2 units	2.6 units	$4 \times 10^{-3}$ u/ $\mu$ l
qPCR Pol (red cap)	0.25 $\mu$ l	3.25 $\mu$ l	0.025 u/ $\mu$ l
PCR-grade water	Fill up to 45 $\mu$ l	Fill up to 585 $\mu$ l	

1) The optimal concentration of each primer may vary from 100 to 500 nM.

2) UNG (Uracil-N-Glycosylase) can be used to prevent carry-over contaminations of DNA from previous PCR reactions. UNG is not provided by this kit.

**Optimization of MgCl<sub>2</sub> concentration**

 A concentration of 3.0 mM Mg<sup>2+</sup> as provided by the 10x qPCR Buffer complete (green cap) is recommended for most applications. For an individual optimization use the reaction buffer without MgCl<sub>2</sub> and add MgCl<sub>2</sub> Stock Solution as shown in the table below.

per 50 $\mu$ l PCR reaction				
MgCl <sub>2</sub> Stock Sol.	4 $\mu$ l	6 $\mu$ l	8 $\mu$ l	12 $\mu$ l
Final MgCl <sub>2</sub> conc.	2 mM	3 mM	4 mM	6 mM

**Dispensing the master mix**

 Vortex the master mix thoroughly to assure homogeneity. Dispense 45  $\mu$ l to a PCR tube or each well of the PCR plate.

**Addition of template DNA**

 Add 5  $\mu$ l of sample template DNA to each reaction vessel containing 45  $\mu$ l master mix and cap or seal the tube / plate. Do not exceed 500 ng DNA per 50  $\mu$ l reaction as final concentration. Tubes or plates should be centrifuged before cycling to remove possible bubbles.

**Recommended cycling conditions**

UNG treatment <sup>3)</sup>	50 °C	2 min	1x
Initial denaturation and polymerase activation	95 °C	2 min	1x
Denaturation	95 °C	15 sec	40-50x
Annealing and elongation	60-65 °C <sup>4)</sup>	30 sec <sup>5)</sup>	

 3) Cycling step 1 is only required if an UNG (Uracil-N-Glycosylase) treatment is applied.  
 4) The annealing temperature depends on the melting temperature of the primers and DNA probe used.  
 5) The elongation time depends on the length of the amplicon. A time of 30 sec for a fragment of up to 500 bp is recommended.

 For optimal specificity and amplification an individual optimization of the recommended parameters, especially of the MgCl<sub>2</sub> concentration and the annealing temperature may be necessary for each new combination of template DNA, primer pair, and DNA probe.

 EvaGreen<sup>®</sup> is a registered trademark and licensed for sale by Biotium, Inc., Hayward, CA, USA  
 SYBR<sup>®</sup> is a registered trademark of Invitrogen Corporation, Carlsbad, California, USA