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Data Sheet (18.02.2010)

mi-real-time EvaGreen® Master

2x ready-to-use hot start mix for real-time PCR with green-fluorescent DNA stain

Cat.-No.	Amount
mi-E2001S	100 rx (50 µl)
mi-E2001L	500 rx (50 µl)

Only for *in vitro* use!
For research only!

2x 1.25 ml/ 10x 1.25 ml
for 100/ 500 rx of 50 µl final volume

2x mi-real-time EvaGreen® Master (blue cap)

- Taq Polymerase: 0.05 u/ µl
- dNTPs (dATP, dCTP, dGTP, dUTP) (200 µM)
- reaction buffer with KCl and $MgCl_2$ (3 mM)
- EvaGreen®, stabilizers

PCR-grade water (white cap)

Storage

Store at -20 °C, avoid frequent thawing and freezing. Storage at 4 °C for up to 3 months possible. mi-real-time EvaGreen® Master must be stored in the dark.

Description

mi-real-time EvaGreen® Master is designed for the quantitative real-time analysis of DNA samples using the fluorescent DNA stain EvaGreen®. The fluorescent dye in the master mix 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. It provides an easy to-handle and 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 Master contains all reagents required for qPCR (except template and primer) in a premixed 2x concentrated ready-to-use solution. The high specificity and sensitivity of the mix 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 formations at low temperatures during PCR setup.

The 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.

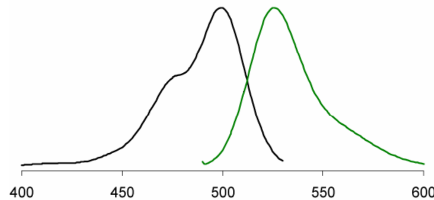
EvaGreen® Fluorescent DNA Stain

EvaGreen® Fluorescent DNA Stain is a superior DNA intercalator dye specially developed for DNA analysis applications including real-time PCR (qPCR) and highresolution 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.

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® Green I dye.

Spectroscopic data

Excitation maximum: Ex = 500 nm (bound to DNA)
Emission maximum: Em = 530 nm (bound to DNA)



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

To perform the EvaGreen-based assay simply select the optical setting for SYBR® 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 exept template as specified. A reaction volume of 20-50 µl is recommended for most real-time instruments. Pipet with sterile filter tips and minimize the exposure 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 µl**	**Final conc.**
2x real-time EvaGreen Master (blue cap)	25 µl	1x conc.
Primer forward (10 µM) [1]	1.5 µl	300 nM
Primer reverse (10 µM) [1]	1.5 µl	300 nM
PCR- grade water	Fill up to 45 µl	

[1] The optimal concentration of each primer may vary from 100 to 500 nM.
If a treatment with UNG (Uracil-N-Glycosylase) is necessary, add 0.2 units of UNG to the master mix (final conc. 4x 10^{-3} u/ µl). UNG is not provided in this kit.

Dispensing the master mix

Vortex the master mix thoroughly to assure homogeneity. Dispense 45 µl to a PCR tube or each well of the PCR plate.

Addition of template DNA

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

Recommended cycling conditions

Initial denaturation and polymerase activation	95 °C	2 min	1x
Denaturation	95 °C	15 sec	30-40x
Annealing [2]	55-65 °C	20 sec	
Elongation [3]	72 °C	30 sec	

[2] The annealing temperature depends on the melting temperature of the primers used.
[3] 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.
If an UNG (Uracil-N-Glycosylase) treatment is applied, start with one cycling step at 50 °C for 2 min.

For optimal specificity and amplification an individual optimization of the recommended parameters, especially of the annealing temperature may be necessary for each new combination of template DNA and primer pair.

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SYBR® is a registered trademark of Invitrogen Corporation, Carlsbad, California, USA

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