

ENCODE 2x Master Mix Green

Cat. No: EIL- 2XMASTERMIX
40 reactions (20ul)

	ENCDE Master Mix Green	ROX internal reference dye 200 uM
Color code	Amber	Amber
Content	1 x 0.4 ml	1 x 0.05 ml

KEY FEATURES:

- All-in-one optimized master mix, including green dye
- High sensitivity
- High efficiency and specificity
- Wide dynamic
- Hot start capacity for room temperature setup

Detection limit: approximately 1 copy

QUANTITATION LIMIT: approximately 24 copies (0.08 ng of human gDNA, correlating to 12 diploid genomes, with 2 gene copies per diploid genome)

Compatibility: All-real time PCR instruments

Introduction:

Quantitative PCR is an important tool for SNP, gene expression analysis and quantification of specific genes. Two general fluorescent chemistries exist for quantitative detection of genes: probes (e.g. TaqMan®, Scorpions™ probes, molecular beacons) and DNA-binding fluorescent dye (e.g. ethidium bromide, SYBR® green, EvaGreen®, PicoGreen®). Encode offers the Encode 2x Masters mix in two formulations: for probe and

including DNA-binding fluorescent dye, making them ideal for most quantitative PCR application.

The ENCODE 2x Masters Mix Green is a single tube 2x reagent including all components necessary to perform DNA-binding dye based real-time PCR amplification. Just add your primers and DNA.

The TEMPase Hot Start DNA Polymerase included in the Encode 2x Master Mix Green improves the specificity of a PCR reaction by increasing amplification of the desired DNA target and decreasing amplification of nonspecific background. TEMPase Hot Start DNA polymerase is a modified Taq DNA polymerases with hot start capabilities.

The ENCODE 2x Master Mix Green solution provides optimal performance on most of the commonly used real-time PCR instruments.

Composition of ENCODE 2x Master Mix Green:

- Optimized buffer system including, TEMPase Hot Start DNA polymerase, dNTPs and fluorescent dye
- ROX internal reference dye provided in a separate tube

Recommended Storage and stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.

Option: Store at +4 °C for up to 3 months.

Quality Control

TEMPase DNA Polymerase is tested for contaminating activities, with no traces of



endonuclease activity, nicking activity or exonuclease activity. The ENCODE 2x Master Mix Green is functionally tested for efficiency and absence of contaminating human genomic DNA.

Pre-protocol Considerations

PCR Primers

It is important - especially in fluorescent DNA dye based quantitative PCR applications - to minimize the formation of non-specific amplification products. Particularly at low target concentration it is important to use the lowest possible primer concentration without compromising the efficiency of the PCR. The optimal concentration of primer pairs is the lowest concentration that results in the lowest C_q and an adequate fluorescence for a given target concentration with minimal or no formation of primer-dimers. The optimal concentrations of upstream and downstream primers are not always of equal molarity. Optimal concentrations of primers are in the range of 100 nM to 500 nM.

Reference Dye

ROX (6-carboxy-X-rhodamine) internal reference dye is included in this kit and serve as an internal reference for normalization of the fluorescent signal when using real time PCR instruments, which can detect ROX. ROX corrects well-to-well variation due to pipetting inaccuracies and fluorescence fluctuations. The presence of ROX does not interfere with qPCR amplification. The excitation and emission of the reference dye are 584 nm and 612 nm, respectively. ROX has direct influence on the ΔR_n amplification plot. Thus, the C_q -value and the amplification plot plateau are influenced by how precisely ROX is added. Therefore. Always be meticulous when pipetting.

Preventing Template Cross-Contamination

Due to the high sensitivity of quantitative PCR there is a risk of contaminating the reactions with the products of previous runs. To minimize this risk, tubes or plates containing reaction products should not be opened or analyzed by gel electrophoresis in the same laboratory area used to set up reactions.

Protocol

Note:

- Prior to the experiment, it is crucial to carefully optimize experimental conditions and to include controls at every stage. See pre-protocol considerations for details.
- Thaw the ENCODE 2x Master Mix. Following initial thawing of the master mix, store the unused portion at +4 °C. **Important:** Multiple freeze-thaw cycles should be avoided. Solutions containing Green DNA dye should be protected from light whenever possible.
- If needed, prepare a fresh dilution of ROX internal reference dye.

The dilution ROX reference dye must be kept in a light protected tube at 4°C.

For a final reaction concentration of 30 nM dilute 1:100 in PCR grade water, 0.5 ul ROX per 50 ul ROX dilution. For a final reaction concentration of 300 nM dilute 1:10 in PCR grade water, 5 ul ROX per 50 ul ROX dilution. Use the dilution as described in table 1.



The use of ROX internal reference dye with a final reaction concentration of 30 nM is recommended for applied Biosystem® 7500, 7500 Fast and ViiA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx.

The use of pf ROX internal reference dye with a final reaction concentration of 300 nM is recommended for applied biosystems® 7500, 7000, 7300, 7700, 7900, 7900 HT, StepOne™ and StepOnePlus™.

1. Prepare the experimental reaction by adding the components in the order shown in table 1.

Table 1. Reaction components (reaction mix and template DNA)

Component	Vol./reaction	Final concentration
ENCODE 2x Master Mix	10 ul	1x
Primer A (10 Uμ)	0.4 ul (0.2-1 ul)	0.2 uM (0.1-0.5 uM)
Primer B (10 Uμ)	0.4 ul (0.2-1 ul)	0.2 uM (0.1-0.5 uM)
ROX 1:100	0.3 ul	30nM-L ROX
ROX 1:10	0.3 ul	300nM-H ROX
PCR-grade H2O	X ul	-
Template DNA	X ul	Genomic DNA: 20 ng (1-100 ng) Plasmid DNA: 0.5 ng (0.1-1 ng) Bacterial DNA: 5 ng (1-10 ng)
TOTAL volume	20 ul	-

* Suggested starting conditions; optimization range in parenthesis

** Optimization of primer concentrations is highly recommended.

2. Gently mix without creating bubbles* (do not vortex).

* Bubbles interfere with detection of fluorescence.

3. Place the reaction in the instrument and run the appropriate program according to the manufacturer's instructions.

Three-step PCR Program

Cycles	Duration of cycle	Temperature
1°	15 minutes	95°C
40	15-30 seconds 30 seconds 30 seconds	95°C 55-65 °C 72 °C

Two-step PCR Program

Cycles	Duration of cycle	Temperature
1°	15 minutes	95 °C
40-50	15-30 seconds 60 seconds	95 °C 55-65 °C

- a. For activation of the TEMPase hot start enzyme.
- b. Denaturation time is varying between thermocyclers.
- c. Set the qPCR instrument to detect and report fluorescence during the annealing/extension step of each cycle.
- d. Choose an appropriate annealing temperature for the primer set used.

Other product sizes, combinations and customized solutions are available. Please ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

