Product Information

Super EvaGreen® Master Mix for HRM (2×)

Catalog Number: S2013

Product Size: 100T, 500T

Contents:

Component	100T	500T
A. 2 × Master Mix	1 mL	5×1 mL
B. 10 × ROX reference dye	0.5 mL	1 mL

Component A contains Super EvaGreen dye, dNTP, PCR buffer (including Tris and $MgCl_2$) and hot-start Taq polymerase. Component B is 10× Rox reference, which may be required on certain ABI instruments (See protocol below).

Parameters

abs/Em = 500/530 nm (DNA bound)

abs = 471 nm (without DNA)

Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 6 months.

Description

Super EvaGreen® Master Mix for HRM (2×) is a professional kit for real-time quantitative PCR and high-resolution melting curve analysis. Super EvaGreen dye binds to dsDNA via a novel "release-on-demand" mechanism, which permits the use of a relatively high dye concentration in qPCR without PCR inhibition. It can make the binding amount of double-stranded PCR products reach the saturation, so it is called saturated dye and will not produce rearrangement like SYBR Green. Another unique feature of Super EvaGreen® is its safety.

This product contains a high-fidelity hot-start Taq DNA polymerase, which is chemically modified and has no activity at room temperature to avoid amplification of non-specific products. This kit can be used for SNP analysis, unknown mutation gene scanning and methylation detection.

A unique feature of Super EvaGreen® dye is its safety. DNA-binding dyes are inherently dangerous due to their potential to cause mutation. UE's scientists designed Super EvaGreen® dye such that it cannot cross cell membranes, thus preventing the dye from being in contact with genomic DNA in live cells. All other commercial PCR dyes, such as SYBR® Green I, enter into cells in a matter of minutes.

Protocol

Before use, thaw at room temperature and mix well by gentle vortexing. After thawing, the master mix should be kept on ice before use. It can be refrozen for storage.

1. Pipet reaction components into each well according to the table as below:

Reaction component	Amount required per 20 µL reaction	Final concentration
2× Master Mix	10 µL	1×
F, R Primers	× µL each	0.1-0.5 µM each
Template	× µL	See Note

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10× ROX	Optional	See Table1
H_2O	Add to 20 μL	

Note: Template concentration: The amount of DNA template added is usually below 100 ng. Because the copy number of the target gene contained in different kinds of DNA templates is different, gradient dilution may be performed if necessary to determine the appropriate amount of DNA template addition. To ensure optimal amplification efficiency, the cDNA sample to be added should not exceed 10% of the total volume of the PCR reaction.

2. Mix the reaction mixture gently by vortexing and transfer the fixed volume to the PCR tube.

3. Set up the PCR program.You may choose one of the following three protocols, depending on the nature of your amplification and the function of the instrument.

A. Two-step fast cycling protocol

This cycling protocol should be applicable to most amplifications where the primer Tm's are designed to be 60℃. Melt curves may be performed by following instructions provided for your instrument.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation Annealing & Extension	95 ℃ 60 ℃	5 s (Note 1) 30 s	45

Note: 1) Denaturation time: The holding time for denaturation can be lower than 5 seconds, including as low as 0 second, if you have a relatively short amplicon. When the denaturation time is set to "0" in the program, it merely means that the temperature is ramped up to 96℃ and then immediately ramped down with no stay. Setting the time to 5 s will ensures a more robust denaturation for relatively long or high GC amplicons. Instruments with fast ramping capability further add reliability to amplicon denaturation.

B . Three-step fast cycling protocol

This cycling protocol can be used if you would like to have the extension step to be performed at a higher temperature than the annealing step. For example, if you have relatively long primers that tend to anneal non-specifically, carrying out the extension step at a higher temperature can reduce nonspecific amplification. Melt curves may be performed by following instructions provided for your instrument.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation Annealing Extension	95 ℃ 50-60 ℃ 72 ℃	5 s 5 s(See Note 2) 25 s(See Note 3)	45

Notes: 2) Annealing temperature: The annealing temperature should be set at your primer Tm, which should generally be 50-60℃ for optimal result. However, whenever possible, primer Tm (and thus extension temperature) should be designed closer to 60℃ (but still within 50-60℃ range) to minimize the gap between annealing and denaturation temperatures. This way, the temperature ramping will take less time, which in turn facilitates amplification.

3) Extension temperature: Extension at 72℃ is usually more efficient for most amplicons. However, for AT-rich amplicons (>70% AT) or amplicons that have an AT-rich patch, extension at 60℃ usually gives better results.

C. Universal cycling protocol

This cycling protocol can be used on nearly all qPCR instruments. The protocol also may be useful for targets that are relatively difficult to amplify under fast cycling conditions.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	95 ℃	5 min	1
Denaturation Annealing & Extension	95 ℃ 60 ℃	15 s 60 s	45

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Super EvaGreen Dye Characteristics

1. Spectral Characteristics

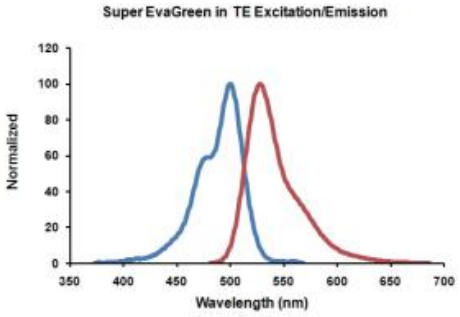

Figure 1. Excitation (left) and emission (right) spectra of Super EvaGreen dye bound to dsDNA in TE buffer

2. Dye stability

Stability comparison of Super EvaGreen and SYBR Green I dyes

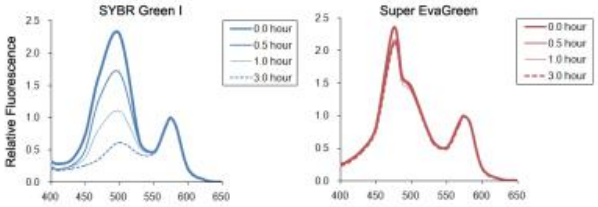

Figure 2. Solutions of Super EvaGreen dye or SYBR Green I at 1.2 μM in pH 9 Tris buffer were incubated at 99 °C. The absorption spectrum of each solution was measured over a period of 3 hours. ROX was added as a stable reference

3. Safety of Super EvaGreen Dye

Comparison of cell membrane permeability of Super EvaGreen® and SYBR Green I fluorescent dyes

Ames testing showed that Super EvaGreen dye is nonmutagenic as well as non-cytotoxic. Super EvaGreen dye appears to be completely cell membrane-impermeable (Figure 3), which may be a key factor responsible for the observed low toxicity. On the other hand, SYBR Green I is known to be a powerful mutation enhancer, possibly by inhibiting the natural DNA repairing mechanism in cells (Ohta, et el. Mutat. Res. 492, 91(2001)). The toxicity of SYBR Green I may be associated with its ability to enter cells rapidly (Figure 3).

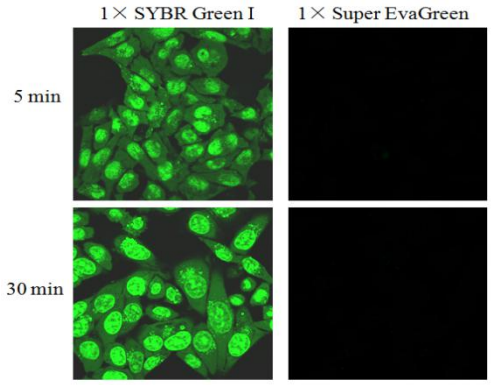

Figure 3. HeLa cells were incubated with SYBR Green I (1.2μM) or Super EvaGreen dye (1.2 μM) at 37°C. Photographs were taken following incubation for 5 min and 30min. SYBR Green I stained cells rapidly while Super EvaGreen appeared to be membrane impermeable.

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Table 1. Recommended ROX Concentration for PCR Instruments

PCR Instrument	Recommended ROX concentration	Amount of 10× ROX per 20 µL Reaction
BioRad: iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, MJ Opticon, Option2, Chromo4, MiniOpticon Qiagen: Rotor-Gene Q, Rotor-Gene3000, Rotor-Gene 6000 Eppendorf: Mastercycler realplex Illumina: Eco RealTime PCR System Cepheid: SmartCyler Roche: LightCycler 480, LightCycler 2.0	No ROX	None
ABI: 7500, 7500 Fast, ViiA 7 Stratagene: MX4000P, MX3000P, MX3005P, QuantStudio, Illumina Eco, Thmorgan Q6,Q4	Low ROX 0.05-0.1× final	Dilute 10× ROX 1:10 with dH_2O to obtain 1× ROX; add 1 to 2 uL of 1× ROX per 20 uL reaction
ABI: 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus	High ROX 1× final	2 uL of 10× ROX per 20 uL reaction

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