Product Information

AugeGreen™ qPCR Master Mix

Catalog Number: S2008

Product Size: 100T, 500T

Contents:

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

The product contains two components. Component A contains AugeGreen™ 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).

Spectral Properties of AugeGreen™ Dye:

The absorption and fluorescence emission spectra of DNA-bound AugeGreen™ dye are very similar to those of SYBR Green I or FAM.

λabs/λem = 500/530 nm (DNA bound)

λabs = 471 nm (without DNA)

Storage and Handling

AugeGreen™ qPCR Master Mix is shipped on blue ice and should be stored immediately upon arrival at -20℃. When stored under the recommended condition and handled correctly, the kit should be stable for at least 12 months from the date of receipt. Before use, thaw the product at room temperature (warm up directly without ice) and gently vortex to mix. Uneven mixing of reagents can result in poor experimental results. After thawing, all experiments should be performed on ice. The product can be refrigerated for storage.

Description

AugeGreen™ qPCR Master Mix is a ready-to-use hot-start mix for qPCR and DNA melt curve analysis of PCR amplicons. It is formulated for qPCR using a fast cycling protocol, but also can be used for qPCR with regular cycling protocols.

AugeGreen™ dye is a unique DNA-binding dye with features ideal for both qPCR and melt curve analysis. AugeGreen™ 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.

AugeGreen™ qPCR Master Mix contains our proprietary chemically-modified UE hot-start Taq DNA Polymerase. Unlike AmpliTaq Gold, which is also a chemically modified Taq but takes 10 minutes or longer to activate, this UE Taq DNA Polymerase is fully activated in 2 minutes with high activity recovery, making it particularly suitable for fast PCR. UE HS-Taq is completely inactive at room temperature and largely free of DNA contamination. This makes UE HS-Taq superior to any antibody-based hot-start Taq, which is typically not completely inactive at room temperature and is prone to DNA contamination due to the nature of antibody production.

A unique feature of AugeGreen™ dye is its safety. DNA-binding dyes are inherently dangerous due to their potential to cause mutation. With this in mind, our scientists designed AugeGreen™ 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

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enter into cells in a matter of minutes. SYBR Green I, for example, has been shown to be environmentally more toxic than ethidium bromide, a well-known mutagen.

An added benefit of AugeGreen™ Master Mix is that you can analyze your PCR product by gel electrophoresis without the need to add another DNA-binding dye to either your loading buffer or gel. The AugeGreen™ dye in the Master Mix can act as a DNA prestain, permitting direct visualization of DNA bands following electrophoresis.

Protocols

General Considerations

1. qPCR instruments: For iCycler users, you do not need to add FAM to your PCR mix as AugeGreen™ dye has a slight background fluorescence that provides adequate and stable baseline level fluorescence. For Roche LightCycler users using glass capillaries for reactions, you need to add BSA to your PCR reactions (~0.5 mg/mL final concentration). BSA is not necessary if transparent plastic capillary tubes are used.

2. Instruments for melt curve analysis: Suitable instruments include Rotor-Gene 6000, ABI 7500 FAST and HR1™, 384-well LightScanner™ and Roche LightCycler 480. Rotor-Gene 6000, ABI 7500 FAST and Roche LightCycler 480 are capable of performing both qPCR and melt curve analysis. Follow the manufacturer's instruction for data collection and analysis.

3. Expected ΔR and ΔR_N: When comparing signal strength among various commercial qPCR Master Mixes, one needs to be mindful of the method used in the comparison. Conventionally, ΔR is the fluorescence gain above the baseline. In general, 10 µL of 1×AugeGreen™ reaction generates higher ΔR than 50 µL 1× PowerSYBR from ABI or 1× SYBR GreenER from Invitrogen. ΔR_N is defined as ΔR divided by the signal in the ROX channel. Therefore, a higher concentration of ROX will generate smaller ΔR_N. ΔR_N will also become smaller when ROX is excited at its maximal as in the case of ABI 7500, iCycler IQ, MJ opticon, MJ Chromo4, MX3000, and MX4000. Accordingly, the lower ROX concentration used in some commercial SYBR Green Master Mixes will produce a higher ΔR_N.

4. Expected kinetic curve: Based on our comparative studies, amplification curves of AugeGreen™ Master Mix generally are more robust than other commercial Master Mixes formulated using SYBR Green I. Because of SYBR's inhibitory effect, SYBR-based Master Mixes may tend to stall amplification 5-7 cycles after the signal reaches the Ct threshold. In contrast, reactions in AugeGreen™ Master Mix can continue to amplify for as many as 50 cycles.

5. Expected Ct value: Under similar conditions, Ct values generated by AugeGreen™ and SYBR Green I may differ from each other by +1 or –1.

6. Amplicon length: To maximize amplification efficiency with AugeGreen™ Master Mix, the optimal amplicon length is 50-200 bp. For longer amplicons you may need to extend the elongation time.

7. Gel electrophoresis analysis of PCR product: To analyze your PCR product by gel electrophoresis using AugeGreen™ dye in the Master Mix as a prestain, simply add DNA loading buffer your PCR reaction solution, load on a gel, and conduct electrophoresis as usual. No additional DNA-binding dye needs to be added to either the loading buffer or the gel. Gel visualization can be carried out using a 254 nm UV box, or a gel imager or Dark Reader using a SYBR Green filter. Alternatively, the gel may be imaged using a 488 nm laser-based gel scanner.

PCR Reaction Setup

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

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Reaction component	Amount required per 20 μL reaction	Final concentration
2×AugeGreen™ Master Mix	10μL	1×
F, R Primers	× μL each	0.1-0.5 μM each
Template	× μL(See Notes 1 & 2)	See Note 3
10× ROX	Optional	See Note4, Table1
H_2O	Add to 20 μL	

Notes

1. cDNA templates: AugeGreen™ Master Mix is suitable for mRNA quantitation if a two-step procedure is followed. The first step involves converting the mRNA to cDNA by reverse transcription (components not provided). A portion of the synthesized cDNA can then be quantitated by using AugeGreen™ kit in the second step. To ensure optimal amplification efficiency, the aliquot of the cDNA sample to be amplified should not exceed 10% of the volume of the PCR reaction. We recommend cDNA synthesis kits US Everbright Inc. (P.R. China, Suzhou). For accurate quantitation of transcript levels, a no-RT control is recommended to check for possible genomic DNA contamination.

2. One-step RT-qPCR can also be applied for mRNA quantitation. Primer sets must be well characterized to ensure no primer-dimer formation. We recommend that you titrate the amount of reverse transcriptase and the duration of the RT step. If possible, design primers to have Tm at 55 ℃, run both RT step and extension step at 55 ℃. For accurate quantitation of transcript levels, a no-RT control is recommended to check for possible genomic DNA contamination.

3. Template concentration: The optimal amount of template DNA varies by application. Recommended amounts of genomic DNA template per reaction typically range from 50 pg to 50 ng per reaction. Recommended amounts of cDNA typically range from 50 fg to 50 pg, based on the amount of input RNA in the RT reaction.

4. ROX reference dye: For certain instruments, ROX is necessary for accurate Ct determination from well to well. Refer to Table 1 (See Page 5-6) for the recommended ROX concentration for your instrument. ROX may add noise to melt curve analysis, which could be mistaken for real peaks. Thus, in case of unexpected peaks, uncheck “ROX” in the “Passive Reference Dye” box in the software so that data is not collected from the ROX fluorescence channel, then re-analyze the data.

Cycling Protocols

You may choose one of the following three protocols, depending on the nature of your amplicon and instrument capability.

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 5) 30 s	45

Note

5. 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 ℃	10 s 10 s(See Note6) 10 s(See Note7)	45

Notes

6. 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.

7. 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 ℃	2 min	1
Denaturation Annealing & Extension	95 ℃ 60 ℃	15 s 60 s	45

AugeGreen™ Dye Characteristics

Spectral Characteristics

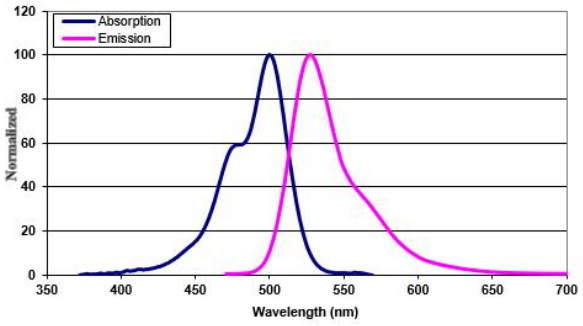

Figure 1. Excitation (left) and emission (right) spectra of AugeGreen™ dye bound to dsDNA in pH 7.3 PBS buffer.

Safety of AugeGreen™ Dye

AugeGreen™ 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. The toxicity of SYBR Green I may be associated with its ability to enter cells rapidly (Figure 2).

Comparison of Cell Membrane Permeability between AugeGreen™ Dye and SYBR Green I

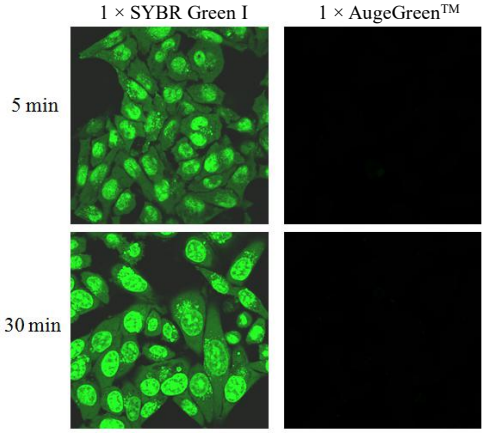

Figure 2. HeLa cells were incubated with SYBR Green I (1.2 μM) or AugeGreen™ dye (1.2 μM) at 37°C. Photographs were taken following incubation for 5 min (panel A) and 30min (panel B). SYBR Green I stained cells rapidly while AugeGreen™ appeared to be membrane impermeable.

For Real Time RT-PCR reactions, the reagents used for the reverse transcription reaction are recommended:

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UEIris II RT-PCR System for First-Strand cDNA Synthesis (no DNase I) (Catalog No. R2027), UEIris II RT-PCR System for First-Strand cDNA Synthesis (with DNase I) (Catalog No. R2028). The above products can be combined with AugeGreen™ qPCR Master Mix to obtain highly reliable results.

Note

This product is for scientific research use only and cannot be used for medical or diagnostic procedures of humans and animals. This product cannot be used as food, cosmetics or household products.

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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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