Product Information

AugeGreen™ , 20× in warter

Catalog Number: S2007

Product Size: 1 mL, 5 mL

Parameters

- Appearance: Light orange solution
- λEx/λEm = 500/530 nm (with DNA)
- λEx = 471 nm (without DNA)

Storage

Store at -20°C and protect from light. When stored as directed, product is stable for at least 12 months.

Description

AugeGreen™ is a DNA-binding dye for real-time quantitative PCR (qPCR). The advantages of this dye make it far superior to SYBR Green I. In addition to similar spectral properties, AugeGreen™ has three main characteristics that distinguish it from SYBR Green I.

First of all, AugeGreen™ has much less inhibition on PCR than SYBR Green I. Therefore, the qPCR experiment using AugeGreen™ can use fast PCR steps. At the same time, AugeGreen™ can use higher concentration in the experiment to obtain much stronger amplified signal than SYBR Green I. Higher concentration of AugeGreen™ also eliminates the defect of "dye redistribution", so that AugeGreen™ can be used not only for multiple PCR, but also for high resolution dissolution curve analysis (HRM).HRM is being increasingly used in post-PCR genotyping and heterologous double-strand analysis. Because SYBR Green I can inhibit PCR, so its concentration must be very low, so SYBR Green I can not solve the problem of dye redistribution caused by low concentration, neither can it be used for multiple PCR nor HRM. At the same time, the problem of dye redistribution may also affect the reliability of the conventional melting curve, because DNA chains with low melting point may not be detected for this reason.

Secondly, AugeGreen™ is very stable. It will not be destroyed during normal storage, operation and PCR process.

Dyes in buffer solutions can be safely stored at room temperature or in refrigerators, or can be repeatedly frozen and thawed. On the contrary, SYBR Green I is unstable and has stronger inhibition on PCR after degradation.

Thirdly, AugeGreen™ is safer than SYBR Green I because it does not have cell membrane permeability. Independent laboratory tests showed that AugeGreen™ had neither mutagenicity nor cytotoxicity. On the contrary, although SYBR Green I itself is very weak in mutagenicity, it may inhibit the repair mechanism of normal DNA in cells and enhance its mutagenicity. Considering the wide use of PCR, its safety should be paid enough attention.

Protocol

1. Set up the PCR reaction (For reference only)

name	volume
10×polymerase buffer without magnesium	5 μL

50 mM MgCl2	2.5 µL
2 mM dNTP	5 µL
20×AugeGreen™	2.5 µL
Taq DNA polymerase	1-5 units
F, R Primers	0.1-0.5 µM each of primers (final concentrations)
DNA	appropriate amount
dH_2O	to a final volume of 50 µL

Note: The amount of DNA added is usually less than 100 ng. Because different kinds of DNA contain different copies of target genes, gradient dilution can be carried out if necessary to determine the appropriate amount of DNA template addition. The amount of cDNA added as template should not exceed 10% of the total volume of the PCR reaction solution.

2. Perform real-time quantitative PCR program to collect fluorescent signals.

US EVERBRIGHT INC.
Tel:0512-88965152
Web:www.useverbright.com/

Instructions

Experimental purpose

Real-time quantitative PCR detection 20×AugeGreen™

Main reagent

1. Prime

hβ-actin：

forward 5’ -CACCCACACTGTGCCCATCTACGA-3’

reverse 5’-CAGCGGAACCGCTCATTGCCAATGG-3’

2. HS Taq DNA Polymerase: Thermo(EP0612)

3. Glycerlo: Sigma(V900090-500 mL)

4. BSA: Sigma(A7030)

5. 10 mM dNTPs: Takara(4019)

Protocol

1. Set up the 2×AugeGreen™ Buffer

2×AugeGreen™ Buffer		
Component	**concentration**	**volume（μL）**
1 M Tris HCl pH8.5	50 mM	5
500 mM $(NH_4)_2SO_4$	20 mM	4
50 mM $MgCl_2$	7 mM	14
50% glycerol	2.5%	5
DMSO	10 %	10
20×AugeGreen™ Buffer	2×	10
10 mM dNTPs	0.4 mM	4
2 % Tween20	0.03 %	1.5
1 mg/mL BSA	22 mg/mL	2.2
H_2O		44.3
Total volume		100

2. Prepare of q-PCR reaction system

Component	volume（μL）
10 μM primers	1μL
DNA	appropriate amount
HS Taq （5 U/μL）	0.2 μL
2×AugeGreen™ buffer	10 μL
H_2O	to a final volume of 20 μL

Note: a.The amount of DNA added is usually less than 100 ng. Because different kinds of DNA contain different copies of target genes, gradient dilution can be carried out if necessary to determine the appropriate amount of DNA template addition. The amount of cDNA added as template should not exceed 10% of the total volume of the PCR reaction solution.

b. The final concentration of primers is generally controlled in the range of 0.1-0.5 μM

3. The experimental groups were divided into three groups: standard control sample pore (positive control), test sample pore and blank control pore (negative control). At the same time, three parallel experiments should be carry out.

4. Centrifugation after mixing. Perform real-time quantitative PCR program.(Roche:LC96)

PCR program:

	temperature	time
step1	95 ℃	2 min
step2	95 ℃	5 sec
step3	60 ℃	30 sec
step2-3 repect for 45 cycles		
Dissolution curve: 57 ℃~99 ℃		

5. Save the date and judge the quality of the samples:

A. Ct value difference between sample and standard

B. Fluorescence intensity difference between sample and standard.

The test results need to be achieved at no significant difference between the above two groups

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