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Sybr Green I

Product Number: ST01

Description

Sybr Green I, is a very sensitive dye for the detection of double stranded DNA (dsDNA), So it has been widely used in non-specific detection of amplification in realtime qPCR experiments. The double-strand DNA-specific Sybr Green I fluorescent reporter offers distinct advantages. Sybr Green I dye is inexpensive, easy to use, and sensitive. Well-designed primers must be used in SYBR Green quantitative RT-PCR reactions because Sybr Green I dye will detect nonspecific products, resulting in an overestimation of the target concentration.

Protocol

1. The flowing table 1 is our Sybr Green I RT-PCR Reagents Kit recipe for reference only. Please optimize it by yourself.

Reagent Final	concentration in the mix
dNTP	0.25mM
Tween20	1%
BSA	0.1% vol.
Tris(pH8.4)	50mM
Hotstart Taq DNA polymerase	1.25u per reaction
NH_4Cl	10mM
KCl	20mM
$MaCl_2$	2.5mM
Sybr Green	2X

2. On ice, prepare a 2×master mix containing no DNA, by mixing the components in the following order: water, DMSO, Taq polymerase buffer, dNTPs, $MgCl_2$, Sybr Green, Taq polymerase.
3. Transfer 2x QuantiTect SYBR Green PCR Master Mix to tubes or plates. Add RNase-free water. Mix the individual solutions.
4. Prepare a reaction mix according to Table 2.

   Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the real-time cycler

PCR Reaction Setup: Table 2

DNA	Template DNA (<500 ng/reaction)
SYBR Mix	25.0μL
Primer1	2μl(5uM)
Primer2	2μl(5uM)
ddH_2O	Add to 50.0μL

Program your real-time cycler according to the program outlined in Table 3

PCR initial activation step	Heating cycling
	Number of cycles 40
95 °C 5min	96 °C 10s,
	60 °C 30s