**SYBR Green I for PCR**

**Technical literature is available at:** [**www.mesgenbio.com**](http://www.mesgenbio.com)**. E-mail MesGen Technical Services if you have questions on use of this system: tech@mesgenbio.com**

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**Catalog Number :** MPY6155 **Lot Number :** Refer to vial **Packaging Size :** 1000μL□5000μL□

**Ex (nm) :** 497 **Em (nm) :** 525 **Solvent :** DMSO

**Background**

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.

**Storage instruction**

2-8°C & Protect from light

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 |
| Hot-start Taq DNA polymerase | 1.25 u per reaction |
| NH4Cl | 10mM |
| KCl | 20mM |
| MaCl2 | 2.5mM |
| Sybr Green | 2X |

2. On ice, prepare a 2x master mix containing no DNA, by mixing the components in the following order : water, DMSO, Taq polymerase buffer, dNTPs, MgCl2, Sybr Green, Taq polymerase.

3. Transfer 2x 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 | 25.0 μL |
| Primer 1 | 2ul（5uM) |
| Primer 2 | 2ul（5uM) |
| dd H2O | Added to 50.0 μL |

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 10s60 °C 30s |

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