Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

20×EvenGreen

Product Number: ST06

Shipping and Storage

12 months at 4°C or -20°C and keep from light

Description

20×EvenGreen is a very sensitive dye for the detection of double stranded DNA (dsDNA). The dye is a green fluorescent nucleic acid dye with features that make it useful for non-specific detection of amplification in realtime qPCR experiments.

Compared with the widely used SYBR Green I, 20×EvenGreen dye is generally less inhibitory toward PCR and less likely to cause nonspecific amplification , 20×EvenGreen dye can be used at a much higher dye concentration than SYBR Green I, resulting in more robust PCR signal.

The PCR reaction can be monitored using our existing optical setting for SYBR Green I or FAM on any commercial real-time PCR cycler. The qPCR protocol provided below is for PCR using regular non-hot-start Taq. Use of a hot-start Taq may require some adjustment of PCR buffer composition in terms of ionic strength and pH to best take the advantage of 20×EvenGreen dye. The water soluble solvent such as DMSO or glycerol are frequently added to stabilize master mixes. These components and pH may need to be optimized depending on the enzyme used.

Concentration

20× in Water

Protocol

For convenience, the 25mM concentrated solution maybe diluted 100 times to a 0.25mM solution in either ddH_2O or Tris (10mM, pH 7-9), which may be stored at 4°C.The following step is recommended for use with non-hotstart Taq.

Note: The recommended concentration of 20×EvenGreen dye for qPCR is 1×. The protocol below uses a diluted stock solution of 20×EvenGreen dye in water.

Perform real-time PCR on a thermocycling fluorometer and record the fluorescence signal at the annealing or extension step.

1. Calculate the volumes of reagents required for the reaction.
2. On ice, prepare a 1× master mix containing no DNA, by mixing the components in the following order: water, Taq polymerase buffer, dNTPs, $MgCl_2$, 20×EvenGreen, Taq polymerase, and primers.
3. Transfer master mix to tubes or plates. Add DNA (50ng per reaction).
4. Proceed with amplification according to your instrument manufacturer. Perform real-time PCR on a thermocycling fluorometer and record the fluorescence signal at the annealing or extension step.

Reagent	Final conc.
5μL each of 2mM dNTP	0.2mM each
ddH_2O to a final volume of 50μL	Adjust to final volume
5μL of 10x polymerase buffer without magnesium	1×
0.1-1μM each of primers (final concentrations)	0.1-1μM
1-5 units of Taq DNA polymerase	1-5 units per reaction
2.5μL of 50mM $MgCl_2$	2.5 mM
2.5 μL of 20×EvenGreen	1×

Note

1. Always use positive and negative controls when doing qPCR experiments.
2. The temperature program for the qPCR amplification does not differ from standard PCR program for the given template and

primers.

3. For the detection, FAM or FAM/SYBR channel should be used.
4. When using ABI Sequence Detection Systems, make sure to select NONE for the passive reference under the tab WELL INSPECTOR.
5. BSA may be required if the reaction is run on a Roche LightCycler. A final BSA concentration of 0.5 mg/mL may be sufficient. With SYBR Green, addition of a protein such as BSA results in a fluorescence increase, which provides a background signal that triggers the start of a LightCycler. Because 20×EvenGreen dye is less sensitive to proteins, you may need to adjust the instrument setting (for background fluorescence) so that the instrument will start.
6. For iCycler users, you do not need to add FAM to your PCR mix because 20×EvenGreen has a slight background fluorescence that provides an adequate and stable baseline level fluorescence for well calibration.
7. For chemically-modified Taq, it may be necessary to reduce the KCl concentration and increase the Tris concentration.
8. The optimal Mg^{2+} concentration for PCR with 20×EvenGreen dye is 2.5 mM.
9. Before pipetting, warm up the 20× solution to room temperature and thoroughly mix the solution by vortexing. 20×EvenGreen is highly stable. However, dye may adsorption to the vial during storage. Vortex the vial for a few seconds to make sure the dye is fully dissolved.
10. For best results, a hot-start enzyme should be used. However, buffer formulation may need to be adjusted.