ExCell Bio
依科赛生物

ExCell Bio

Hot Start SYBR Green qPCR Kit

User Manual

Catalog Number	MB000-3011	200 rxn
	MB000-3012	400 rxn
	MB000-3013	2000 rxn

Introduction

This product is specialized for Real Time PCR using SYBR Green I, an intercalating dye. Chemically modified hot start polymerase used by this kit, coupled with optimized buffer system efficiently diminishes non-specific amplification, significantly improving PCR efficiency and producing highly sensitive results.

This product contains optimal concentration of SYBR SYBR Green I in a convenient 2 × reaction mix, easy for use.

Application

This product produces perfect amplification curve with wide exponential region, ensuring accurate quantification and detection of target gene with reproducible and reliable results.

Components

Components	MB000-3011	MB000-3012	MB000-3013
HotStart SYBR Green qPCR Master Mix	2x1.25ml	4 x1.25ml	20 x 1.25ml
Sterial ddH2O	2x 1.5 ml	4 x 1.5 ml	20 x 1.5 ml

Storage and Stability

Storage: Keep in darkness and avoid strong irradiation during storage and preparation.Store at -20℃ for long-term preservation and 4℃ for frequent use.

Transportation: 0-8℃

Protocol

Materials required but not supplied:

DNA template, primers

Example for 25μl reaction:

Mix the Master Mix thoroughly by inversion prior to use.

1. Prepare the PCR reaction mix:

DNA template	1ul
Forward primer (10 μM)	1ul
Reverse primer (10 μM)	1ul
2×HotStart SYBR Green qPCR Master Mix	12.5ul
ddH_2O	9.5ul

Template: 10~1000ng genomic DNA, 1~30ng plasmid DNA or 1~2 ul reverse transcribed cDNA

Hint: This product is highly sensitive for the quantity of template, so inconsistent sample volume would significantly offset the results. Dilute template appropriately prior to preparation of the reaction mix to couteract this undesired effect and improve the reproducibility.

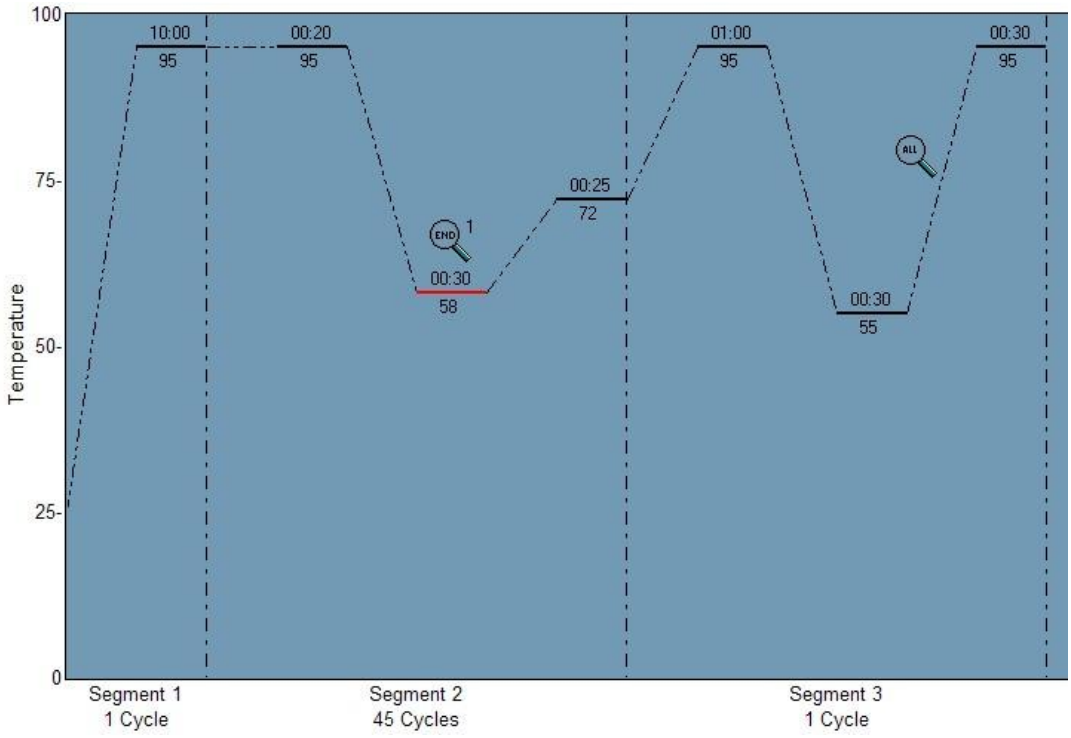
The reaction system could be optimized for specific template and primers to obtain better results and could be adjusted proportionally.

2. Thermocycling

Run a 3-step program in normal case and 2-step program for primers with high T_m.

Heat activation is needed for the DNA polymerase used by this product, which is achieved by incubation for 10min in normal case and 15min for template with high GC content at 94~95℃. Gradient PCR can be performed to determine the optimized annealing temperature.

Recommended program setting is shown below:



Segment 1: Hot start

Segment 2: Thermocycling

Actual annealing time and temperature is determined by T_m and length of the primers.

Actual elongation time is determined by length of the amplicon.

Segment 3: Data collection for melting curve

3. Data analysis

Check the amplification and melting curves and plot the standard curve. Specificity of the PCR could be verified by gel electrophoresis.

Notes

1. For research use only.
2. Free replacement ensured for quality problem.
3. Warranty limited to product value.