YZZY Start DNA polymerase

Instruction manual

Components:

-YZZY Start DNA Polymerase 5U/ul (5000 U)
10x1 ml

Introduction

YZZY Start is a thermostable, recombinant polymerase with aptamer mediated, reversible hot start & immediate activation.

Similar to antibody-based methods, the enzyme is activated by heating. Dropping the temperature below +39°C acts like a switch, shutting off polymerase activity. At temperatures above +45°C, YZZY Start Polymerase is fully activated. ideal for specific priming and fast PCR.

The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction and also exhibits 5'- exonuclease activity.

- High-specificity PCR;
- Routine PCR;
- Real-Time PCR with dual labeled probes and intercalating dyes.

Protocol

Important point before starting:

To reduce the pipetting error when setting several parallel reactions, it is recommended to prepare a common PCR premix containing all the components of the mixture (water, dNTP, buffer, DNA polymerase, primers) with the exception of the DNA template. The volume of PCR premix is calculated based on the number of reactions with the addition of one additional sample.

1. Thaw all reagents, template DNA and primers

2. Prepare a reaction mix according to Table1 (For routine PCR) and Table2 (For Real-Time PCR with Taqman probe).

Table 1. Reaction setup for routine PCR

Component	Volume/ 25 ul reaction	Final concentration
10X Buffer[1]	2,5 ul	1X
Forward primer (10 uM)	0,5 - 1 ul	0,2 - 0,4 uM
Reverse primer (10 мкМ)	0,5 - 1 ul	0,2 - 0,4 uM
dNTPs (10 mM)	0,5 ul	200 uM
$MgCl_2$ (50 mM)[2]	1 – 1,25 ul	2 – 2,5 mM
Template DNA	Variable	0,1 ng - 250 ng (for high complexity genomic DNA)

YZZY Start U/ul	0,1 – 0,2 ul	0,5 - 1 U
Nuclease Free H2O	to 25 ul	

1 - Any standard PCR buffer can be used.

As a starting buffer for optimization, we recommend using a buffer of the following composition 10X AR: 650 mM Tris-HCl (pH 8.8), 166 mM $(NH_4)_2SO_4$, 0.5% Tween 20.

Table 2. Reaction setup for PCR with Taqman probe

Component	**Volume/ 25 ul reaction**	**Final concentration**
10X Buffer[1]	2,5 ul	1X
Forward primer (10 uM)	0,7 - 1 ul	0,28 - 0,4 uM
Reverse primer (10 uM)	0,7 - 1 ul	0,28 - 0,4 uM
Probe (10 uM)	0,25 – 0,7 ul	0,1 – 0,28 uM
dNTPs (10mM)	0,5 ul	200 uM
$MgCl_2$ (50mM)[2]	2 ul	4 mM
Template DNA	Variable	0,1 ng - 250 ng (for high complexity genomic DNA)
YZZY Start 5 U/ul	0,4 ul	2 U
Nuclease Free H2O	to 25 ul	

1 - As a starting buffer for optimization, we recommend using a buffer of the following composition **10X A**: 650 mM Tris-HCl (pH 8.8), 200 mM $(NH_4)_2SO_4$, 0.5% Tween 20.

3. Mix the reaction thoroughly, and dispense appropriate volumes into PCR tubes or plates.

4. Add template DNA to the individual PCR tubes or wells containing the reaction mix.

5. Program your thermocycler according to the program outlined in Table 3 (for routine PCR) and Table 4 (For Real-Time PCR).

Table 3. PCR conditions for routine PCR

Cycle Step	**Tempera-ture**	**Time**	**Num-ber of cycles**
Initial Denaturation	95 ºC	2 min	1
Denaturation	95 ºC	15-30 s	25-35
Annealing	55ºC – 67 ºC	15-30 s	
Elongation	67 ºC	1min/kb	
Final extension	67 ºC	5min	1

The recommended extension temperature is 67°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 67°C is recommended.

Generally, 25–35 cycles yield sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

Table 4 Real-time cycler conditions

Cycle Step	Tempera-ture	Time	Num-ber of cycles
Initial Denaturation	95 °C	2 min	1
Denaturation	95 °C	10 s	45
Annealing	60 °C	15 s + Plate Read	
Elongation	67 °C	15 s	

Shipping and storage

YZZY Start DNA polymerase is shipped at ambient temperature (below 25°C) up to 30 days, at +4 °C for a long time.

YZZY Start DNA polymerase upon arrival should be stored at –20°C, and has a shelflife of 24 months when stored properly under these conditions.

YZZY Start polymerase in the supplied buffer can be stored at room temperature (25 ° C) for 1 month without significant changes in activity and inactivation level.

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