TOROBlue® One HS KOD Kit

Cat No. : KAO-201 Vol: 50μL×40 Reactions

DESCRIPTION

TOROBlue® One HS KOD Kit includes 2× One HS KOD Mix with dye is a ready-to-use premixed solution containing genetically modified hotstart KOD DNA polymerase, PCR buffer, dNTPs,elongation accelerator and loading dye. This mix which enables fast PCR, which has an extension time of 5 sec/ kb by applying genetically modified KOD DNA polymerase and a new elongation accelerator. In addition, this mix provides greater efficiency and elongation capabilities than conventional PCR enzymes. In particular, these show greater amplification success from crude specimens.

FEATURES

-Fast: can amplify the targets using the following very short conditions:0~ 10 kb: 5 sec/ kb ; ≥ 10 kb: 10 sec/ kb
-Easy-to-use: premixed with loading dye for directly loading the gel of Post-Staining or Precasting .
-High Fidelity: exhibits approximately 80-fold higher fidelity than Taq DNA polymerase for cloning.
-Direct PCR: effective for amplification from crude samples.

APPLICATIONS

-Direct PCR
-Colony PCR
-Amplification of NGS libraries
-Site direct gene mutation

COMPONENTS

The kit includes the following reagents, which can be used for 40 reactions for a total 50ul reaction volume, or 100 reactions for a total 20ul reaction volume.
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2× One HS KOD Mix with Dye	1ml ×1 tube

PROTOCOL

1. Standard reaction setup

(1)Before preparing the mixture, all components should be completely thawed on ice.

Component	Reaction volume	Final Concentration
PCR grade water	XμL	
2× One HS KOD Mix with Dye	25μL	1 ×
10uM Forward primer	1.5μL	0.3μM
10uM Reverse primer	1.5μL	0.3μM
Template DNA	YμL	Genomic DNA：≤200 ng / 50μL Plasmid DNA：≤50 ng / 50μL cDNA：≤750 ng / 50 μL Crude Sample：≤5ul / μL
Total Volume	50μL	

(2)Gently mix the reaction solutions and spin down in microcentrifuge.

Notes:
-Primers should be 22-35 bases with Tm ≥ 65°C.
-Optimal primer concentration is 0.3uM. In the case of long targets (≥10 kb), reduced primers concentration (0.15uM) may give more effective amplification.
-When PCR yield is low, increased primers concentration (0.5 uM) may give more effective amplification.
-Decreased the amount of template DNA when no PCR Product.

2. Cycling conditions

[3-step cycle]

Predenaturation : 95°C, 30 sec.
Denaturation : 98°C, 10 sec.
Annealing : (Tm -5) °C, 5 sec.
Extension : 68°C, 5-10sec. /kb
25-45 cycles

[2-step cycle]

Predenaturation :95°C, 30 sec.
Denaturation : 98°C, 10 sec.
Extension : 68°C, 5 -10sec. /kb
25-45 cycles

Notes:
- Extension time:0-10kb,5sec/kb; ≥ 10 kb,10sec/kb.
-Longer extension time may enhance efficiency. For amplification from a low copy DNA or crude sample, the extension time should be 10 sec. /kb.
-Poor amplification may be improved by changing the denaturation step to 94°C, 15sec.

3. Electrophoresis

(1)Directly loading the PCR products and the suitable TOROBlue® Loading Marker into the Post-Staining or Precasting agarose gel.
(2)Run on agarose electrophoresis to detect PCR products and marker.

RELATED PRODUCTS

→Loading Marker：
BDL-029 TOROBlue® 100bp DNA loading Marker
BDL-037 TOROBlue® 1.5kb DNA loading Marker
BDL-036 TOROBlue® 5000bp DNA loading Marker
BDL-031 TOROBlue® 1kb DNA loading Marker
BDL-200 TOROBlue® DL2000 DNA loading Marker
→Nucleic Acid Staining Dye：
RSD-105 TORORed® Nucleic Acid Staining Dye
GSD-105 TOROGreen® DNA Staining Dye
→Reagents for Electrophoresis：
AGR-100 Agarose
TBE-054 5×TBE Buffer
→Instruments for Electrophoresis：
MINI-300 Mini Pro 300V Power Supply
MJ-105A Mini Horizontal Gel Electrophoresis System
MBE-300 Mini BluView Transilluminator

STORAGE

This kit should be kept at -20°C.