



TOROGreen® One HS KOD Kit

Cat No. : KAO-101 Vol: 50µL×40 Reactions

DESCRIPTION

TOROGreen® One HS KOD Kit includes two components, 2× One HS KOD Mix and TOROGreen® 6×DNA Loading Buffer. 2× One HS KOD Mix is a ready-to-use premixed solution containing genetically modified hotstart KOD DNA polymerase, PCR buffer, dNTPs, elongation accelerator. 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. TOROGreen® 6×DNA loading buffer contains the fluorescence dye, which is directly detected on 470nm LED transilluminator after the DNA electrophoresis.

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: offer TOROGreen® 6×DNA loading buffer premixed with fluorescent dye for directly observed on 470nm LED transilluminator after electrophoresis.
- 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 1ml ×1 tube
- TOROGreen® 6×DNA loading buffer 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	25µL	1 ×
10uM Forward primer	1.5µL	0.3µM
10uM Reverse primer	1.5µL	0.3µM
Template DNA	YµL	<ul style="list-style-type: none"> 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 0.3µM. In the case of long targets (≥10 kb), reduced primers concentration (0.15µM) may give more effective amplification.
- When PCR yield is low, increased primers concentration (0.5 µM) 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. ← 25-45 cycles
 Annealing : (Tm -5) °C, 5 sec.
 Extension : 68°C, 5-10sec. /kb

[2-step cycle]

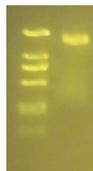
Predenaturation :95°C, 30 sec.
 Denaturation : 98°C, 10 sec. ← 25-45 cycles
 Extension : 68°C, 5 -10sec. /kb

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)Add 1 volume of TOROGreen® 6×DNA loading buffer to 5 volumes of PCR products.
- (2) Mix well, spin down and load the mixture and the suitable TOROGreen® Loading Marker
- (3) Run on agarose electrophoresis to detect PCR products and marker .No additional dye is required for the PCR products and marker.
- (4) Realtime observation of electrophoresis is also possible if a 470nm blue light source is fitted to the electrophoresis tank.
- (5)Use the 470nm blue-light transilluminator or imaging systems photograph the gel.



- 2% agarose gel is recommended.
- 1×TBE buffer is recommended.
- Recommended voltage: 4-10V/cm
- GDL2000 Marker: 1-3µl per loading
- PCR products: 1-3µl per loading

STORAGE

This kit should be kept at -20°C.