

Cat #: 130



 Amount: 125 μl enyzme
 POLYME

 2 x 1.25 ml tubes of 5X KT-PCR Mix
 (sufficient for 500 x 25 μl reactions)

 Shipping conditions: Ice Pack
 Storage conditions: Ice Pack

 Storage conditions: For best performance, store at -20°C
 Thermostability: Retains at least 85% activity after 1 hour at 95°C

 Shelf life: At least 1 year if stored at -20°C and 10 freeze/thaws or at least 1 month if stored at 4°C.

## **PRODUCT DESCRIPTION:**

Our PCR kit contains 5X concentrated master mix (5X KT-PCR Mix) lacking only the enzyme. The enzyme is provided in a separate vial, which allows an adjustment of its final concentration in PCR.

Klentaq LA is an enzyme mixture which allows for longer and more accurate amplification. This kit can be used for conventional as well as real-time PCR. For real-time applications you may need to add a fluorescent dye as an alternative to probes. LA enzymes are not recommended for use with dUTP. 5X KT-PCR Mix composition is: 250 mM Tris-Cl pH 9.2, 80 mM ammonium sulfate, 0.25% Brij 58, 17.5 mM Magnesium Chloride, and 1 mM each dNTP.

### **TYPICAL PCR PROTOCOL for a 25 ul reaction:**

Reagent	Volume	Final Concentration
5X KT-PCR Mix	5 μl	1x
Left Primer	variable	200 nM
Right Primer	variable	200 nM
DNA template†	variable	0.1-100 ng
PCR Enhancer Cocktail (recommended)*	12.5 µl	1x
Klentaq LA	0.05 – 0.25 µl **	
De-ionized distilled H2O	Adjust final volume to 25 ul	

† DNA amount depends mostly on genome size and target gene copy number.

\* For optimal performance, we recommend using one of our PCR Enhancer Cocktails (PEC-1, PEC-1GC, PEC-2, or PEC-2-GC) or 1.3 M Betaine, a general PCR enhancer.

\*\* To determine specific optimal enzyme concentration, we strongly recommend an enzyme titration test for each target. Good starting amount of the enzyme per 25 ul reaction is 0.05 ul. Targets larger than 1 kb may require more enzyme.

#### **CYCLING CONDITIONS:**

- 1. Denaturing:  $94^{\circ}$  for 2 minutes for 1 cycle
- 2. Denaturing:  $94^{\circ}$  for 40-60 seconds
- 3. Annealing:  $50^{\circ}-68^{\circ}$  depending on the specific primers (5° less than Tm) for 40-60 seconds
- 4. Extension: 68° for 2 min/kb target
- 5. Repeat steps 2-4 for 25-40 cycles

#### Please visit us on the web at www.klentaq.com for troubleshooting and detailed protocols.

# **REFERENCES:**

Barnes, W.M. (1994) PCR amplification of up to 35 kb DNA with high fidelity and high yield from bacteriophage templates, PNAS 91, 2216-2220.