5x Hot Start ZipTaq PCR Kit Cat #: HS324

Amount: 125 ul enyzme
2 x 1.25 ml tubes of 5X ZT-PCR Mix
(sufficient for 500 x 25 µl reactions)
Shipping 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.
Expiration: On tube label

PRODUCT DESCRIPTION:
Our PCR kit contains 5X concentrated master mix (5X ZT-PCR Mix) lacking only the Hot Start ZipTaq enzyme. The enzyme is provided in a separate vial, which allows for adjustment of its final concentration in the PCR.

ZipTaq DNA Polymerase is a Taq polymerase with multiple mutations that make it the fastest enzyme in our collection. With the addition of one of our PECs, reactions can tolerate up to 40% blood. It is provided here with aptamer based technology, enabling room temperature reaction set-up. 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. Concentration of dNTPs is 1mM each.

TYPICAL PCR PROTOCOL for a 25 µl reaction:

Reagent	Volume	Final Concentration
5x ZT-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)*		
Hot Start ZipTaq	0.05 – 0.25 µl **	
De-ionized distilled H2O	Adjust final volume to 25 µl	

† 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. A good starting amount of the enzyme per 25 µl reaction is 0.05 µl for purified DNA templates and 0.25 µl for crude samples containing 5-10% whole blood, plasma or serum. Targets larger than 1 kb may require more enzyme.

3-STEP CYCLING CONDITIONS (For 25 ul reactions):
1. Initial Denature: 95° for 1 minutes for 1 cycle *
2. Denaturing: 94° for 1-5 seconds †
3. Annealing: 50°-68° depending on the specific Tm primers for 1-5 seconds †
4. Extension: 68° for 1-5 seconds/kb target †
5. Repeat steps 2-4 for 25-40 cycles

2-STEP CYCLING CONDITIONS (For 25 ul reactions):
1. Initial Denature: 95° for 1 minutes for 1 cycle *
2. Denaturing: 94° for 1-5 seconds †
3. Annealing/Extension: 60°-65° depending on the specific Tm primers for 1-5 seconds/kb target †
4. Repeat steps 2 and 3 for 25-40 cycles

* A 2-5 minute initial denaturation is recommended for crude samples containing 5-10% whole blood, plasma or serum.
† Exact number of seconds will depend on the thermocycler and target. We recommend experimentation to determine precise cycling parameters.

Please visit us on the web at www.klentaq.com for data, troubleshooting and related products