PCRBIOSYSTEMS
simplifying research

# PCRBIO HS Taq 500units/µl

## Product description:

PCRBIO Taq (500u/µl) is a robust enzyme for all your everyday PCR applications including genotyping, screening and diagnostics. Glycerol, detergents and many PCR enhancers are often prohibitive for successful lyophilisation. The use of a highly concentrated enzyme preparation ensures that components prohibitive to lyophilisation are diluted out. The hot start (HS) additive is supplied in 10mM Tris pH 8.0, it does not contain glycerol.

PCRBIO Taq (500u/µl) can perform consistently well on a broad range of templates (including both GC and AT rich). PCRBIO Taq DNA Polymerase has 5'-3' exonuclease activities, but no 3'-5' exonuclease (proofreading) activity. The enzyme has the same error rate as wild-type taq DNA polymerase, approximately 1 error per 2.0 x $10^5$ nucleotides incorporated. PCR products generated with PCRBIO Taq DNA Polymerase are A-tailed and may be cloned into TA cloning vectors. PCRBIO Taq (500u/µl) is a lyophilisation ready polymerase for incorporation into diagnostic kits and point of care diagnostic devices.

| Component | 2000 units | 20000 units | 200000 units |
| --- | --- | --- | --- |
| PCRBIO Taq (500u/µl) | 1x 4µl | 1x 40µl | 1x 400µl |
| HS Additive (4µg/µl) | 100 µl | 1mL | 10mL |

## Shipping and Storage

On arrival the kit should be stored at -20°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

## Limitations of product use

The product may be used only for in vitro research purposes.

## Technical support

For technical support and troubleshooting please email technical@pcrbio.com the following information:

Amplicon size
Reaction setup
Cycling conditions
Screen grabs of gel images

www.pcrbio.com

## Important considerations

Enzyme Concentration: For fast PCR we recommend using between 1 and 5units of PCRBIO Taq DNA polymerase per 50µl reaction.

Hotstart Additive: For optimal hotstart the ratio of HS additive to PCRBIO Taq must be 200ng HS additive/ Taq unit. The hotstart additive is supplied in 10mM Tris-HCl, pH8.0. The additive is ready to lyophilise, it does not contain glycerol.

Template: For eukaryotic DNA use between 5ng and 500ng per reaction, for cDNA use below 100ng per reaction.

Primers: Primers should have a predicted melting temperature of around 60˚C, using default Primer 3 settings (http://frodo.wi.mit.edu/primer3/). The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

Annealing: We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 55˚C annealing temperature then increase in 2˚C increments if non-specific products are present.

Extension: Optimal extension is achieved at 72˚C. The optimal extension time is dependent on amplicon length and complexity of template. 15 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons between 1kb and 6kb. For shorter amplicons a 1 second extension is sufficient.

10x Reaction buffer: We suggest preparing 10x reaction buffer (100mM Tris-HCl pH8.3 @ 25 ˚C, 500mM KCl, 30mM $MgCl_2$, 10mM dNTPs, 1.5% preferred detergent). Filter sterilize.

Enhancers: Standard PCR enhancers may be added to the reaction.

## Reaction setup

1. Prepare a master mix based on the following table:

| Reagent | 50µl reaction | Final concentration | Notes |
|---|---|---|---|
| 10x Reaction buffer | 5.0µl | 1x | Prepared as above |
| Forward primer (10µM) | 2.0µl | 400nM | See above for optimal primer design |
| Reverse primer (10µM) | 2.0µl | 400nM | |
| Template DNA | <100ng cDNA, <500ng genomic | variable | See above for template considerations |
| PCRBIO Taq DNA polymerase (500u/µl) | 0.002µl to 0.01µl | 1 to 5u per reaction | |
| HS Additive (4µg/µl) | 0.05µl to 0.25µl | Use 200ng per unit | Correct ratio of HS additive:Taq is essential for optimal hotstart |
| PCR grade $dH_2O$ | Up to 50µl final volume | | |

2. Cycle using conditions based on the following table:

| Cycles | Temperature | Time | Notes |
|---|---|---|---|
| 1 | 95˚C | 1min | Initial denaturation |
| 40 | 95˚C<br>55˚C to 65˚C<br>72˚C | 15 seconds<br>15 seconds<br>1 to 90 seconds | Denaturation<br>Anneal<br>Extension (15 seconds per kb) |