# MyTaq™ HS Red DNA Polymerase

Shipping: On dry / blue ice

Catalog numbers:

BIO-21114 : 250 Units

Batch No.: See vial

BIO-21115 : 1000 Units

Concentration: 5U/μL

BIO-21116 : 2500 Units

**Store at –20°C**

BIOLINE

A Meridian Life Science® Company

**Storage and stability:**
The MyTaq HS Red is shipped on dry/blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided.

**Expiry:**
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

**Safety precautions:**
Please refer to the material safety data sheet for further information.

**Unit definition:**
One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72 °C.

**Notes:**
Research use only.

## Description

MyTaq™ HS Red DNA Polymerase is a high performance PCR product that is powered by antibody-mediated hot-start, specifically designed for fast, highly-specific, hot-start PCR. MyTaq HS Red does not possess polymerase activity during the reaction set-up, thus reducing non-specific amplification including primer-dimer formation. The advanced formulation of MyTaq HS Red allows fast cycling conditions, considerably reducing the reaction time without compromising PCR specificity or yield. This new enzyme from Bioline is supplied with 5x MyTaq buffer system, a proprietary formulation that saves time and delivers superior results, as it contains dNTPs, $MgCl_2$ and enhancers at optimal concentrations which removes the need for optimization.

The specially designed MyTaq Red formulation does not interfere with the PCR and enables users to load samples directly onto a gel after the PCR without the need to add loading buffer.

MyTaq HS Red only requires the addition of template, primers and water, reducing the risk of pipetting errors and contamination as well as reducing the set-up time.

## Components

| | 250 Units | 1000 Units | 2500 Units |
|---|---|---|---|
| MyTaq HS Red DNA Pol | 1 x 50 μL | 1 x 200 μL | 2 x 250 μL |
| 5x MyTaq Red Reaction Buffer | 2 x 1 mL | 8 x 1 mL | 14 x 1.5 mL |

## Standard MyTaq HS Red Protocol

The following protocol is for a standard 50μl reaction and can be used as a starting point for reaction optimization. Please refer to the Important Considerations and PCR Optimization section.

**PCR reaction set-up:**

| | |
|---|---|
| 5x MyTaq Red Reaction Buffer | 10 μL |
| Template | as required |
| Primers 20 μM each | 1 μL |
| MyTaq HS Red DNA Polymerase | 1 μL |
| Water ($ddH_2O$) | up to 50 μL |

**Standard PCR cycling conditions:**

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 95 °C | 1 min | 1 |
| Denaturation | 95 °C | 15 s | 25-35 |
| Annealing* | User determined | 15 s | |
| Extension* | 72 °C | 10 s | |

* These parameters may require optimization, please refer to the PCR optimization section if needed.

## Colony PCR Protocol

MyTaq HS Red can be used for amplification of plasmid DNA directly from liquid cultures or from colonies on agar plates:

- From liquid culture: up to 8 μL of the overnight culture can be directly added to the final reaction mix.
- From colonies: we recommend using a sterile tip to stab the colony and resuspend it directly in the 50 μL reaction mix.

Recommended cycling conditions for colony PCR of fragment up to 1 kb.

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 95 °C | 1 min | 1 |
| Denaturation | 95 °C | 15 s | 25-35 |
| Annealing* | User determined | 15s | |
| Extension* | 72 °C | 10 s | |

* These parameters may require optimization, please refer to the PCR optimization section if needed.

## Multiplex PCR Protocol

MyTaq HS Red is suitable for multiplex PCR but adjustment of the annealing temperature and extension time may be required.

- Annealing temperature: We suggest using 55 °C as a starting annealing temperature. If further optimization is required we recommend using a temperature gradient to determine the optimal annealing temperature needed for the multiplex PCR.
- Extension time: since multiplex PCR require generally longer extension step, we suggest starting with a minimum of 90 s and increasing it if required.

Recommended cycling conditions for multiplex PCR of fragment up to 1 kb.

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Initial denaturation | 95 °C | 1 min | 1 |
| Denaturation | 95 °C | 15 s | 25-35 |
| Annealing* | User determined | 15 s | |
| Extension* | 72 °C | 90 s | |

* These parameters may require optimization, please refer to the PCR optimization section if needed.

## Important Considerations and PCR Optimization

The optimal conditions will vary from reaction to reaction and are dependent on the template/primers used.

**5x MyTaq Reaction Buffer:** The 5x Reaction Buffer comprises of 5 mM dNTPs, 15 mM $MgCl_2$, stabilizers and enhancers. The concentration of each component has been extensively optimized, reducing the need for further optimization. Additional PCR enhancers such as Betaine etc. are not recommended.

**Primers:** Forward and reverse primers are generally used at the final concentration of 0.2-0.6 μM each. As a starting point, we recommend using 0.4 μM final concentration (*i.e.* 20 pmol of each primer per 50 μL reaction volume). Too high a primer concentration can reduce the specificity of priming, resulting in non-specific products.

When designing primers we recommend using primer-design software such as Primer3 (http://frodo.wi.mit.edu/primer3) or visual OMP™ (http://dnasoftware.com) with monovalent and divalent cations concentrations of 10 mM and 3 mM respectively. Primers should have a melting temperature (Tm) of approximately 60 °C

**Template:** The amount of template in the reaction depends mainly on the type of DNA used. For templates with low structural complexity, such as plasmid DNA, we recommend using 50 pg-10 ng DNA per 50 μL reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200 ng DNA per 50 μL reaction, this can be varied between 5 ng-500 ng. It is important to avoid, using template resuspended in EDTA-containing solutions (*e.g.* TE buffer) since EDTA chelates free $Mg^{2+}$.

**Initial Denaturation:** The initial denaturation step is required to activate the enzyme and fully melt the template. We recommend 1 minute of initial denaturation at 95 °C, however for more complex templates such as eukaryotic genomic DNA, longer initial denaturation times of up to 3 minutes might be required.

**Denaturation:** Our protocol recommends a 15 s cycling denaturation step at 95 °C which is also suited to GC-rich templates. For low GC content (40-45%) amplicons, the denaturation step can be decreased down to 5 s, however for templates containing high GC content of >55%, a longer denaturation time of 15 s is recommended.

**Annealing temperature and time:** The optimal annealing temperature is dependent upon the primer sequences and is usually 2-5 °C below the lower Tm of the pair. We recommend starting with a 55 °C annealing temperature and, if necessary, to run a temperature gradient to determine the optimal annealing temperature. Depending on the reaction the annealing time can also be reduced down to 5 s.

**Extension temperature and time:** The extension step should be performed at 72 °C. The extension time is dependent on the length of the amplicon and the complexity of the template. An extension time of 10 s is recommended for amplicons up 1 kb or up to 5 kb in the case of a low complexity template such as plasmid DNA. For amplification of fragments over 1 kb from high complexity template, such as eukaryotic genomic DNA, longer extension times are recommended. In order to determine the fastest cycling protocol, we suggest increasing the extension time up to 30 s/kb.

## Troubleshooting Guide

| Problem | Possible Cause | Recommendation |
|---|---|---|
| **No PCR product** | Missing component | - Check reaction set-up and volumes used |
| | Defective component | - Check the aspect and the concentrations of all components as well as the storage conditions. If necessary test each component individually in controlled reactions |
| | Enzyme concentration too low | - Increase enzyme quantity to up to 5U/50 μL reaction |
| | Cycling conditions not optimal | - Decrease the annealing temperature<br>- Run a temperature gradient to determine the optimal annealing temperature<br>- Increase the extension time, especially if amplifying long target<br>- Increase the number of cycles |
| | Difficult template | - Increase the denaturation time |
| **Smearing or Non-Specific products** | Excessive cycling | - Decrease the number of cycles |
| | Extension time too long | - Decrease the extension time |
| | Annealing temperature too low | - Increase the annealing temperature |
| | Primer concentration too high | - Decrease primer concentration |
| | Contamination | - Replace each components in order to find the possible source of contamination<br>- Set-up the PCR and analyze the PCR product in separated areas. |

## Technical Support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact your local distributor or our Technical Support with details of reaction set-up, cycling conditions and relevant data.

Email: tech@bioline.com

## Associated Products

| Product Name | Pack Size | Cat. No. |
|---|---|---|
| Agarose | 500 g | BIO-41025 |
| Agarose tablets | 300 g | BIO-41027 |
| HyperLadder™ 1kb | 200 Lanes | BIO-33025 |



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