# Glycerol-Free HS Tth DNA Polymerase

# Product Handling Guide

| | |
|---|---|
| Shipping: | On Dry/Blue Ice |
| Catalog number: | MDX205 |
| Batch No.: | See vial |
| Concentration: | 200 U/µL |

**Store at –20 °C**

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**Storage and stability:**
Glycerol-Free HS Tth DNA Polymerase is shipped on dry/blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided. Thawing during transportation does not affect the product performance. Solutions should be mixed/equilibrated after each thawing to avoid phasing.

**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:**
Read and understand the SDS (Safety Data Sheets) before handling the reagents. Hardcopies of the SDS will be provided with the first shipment, thereafter they will be available upon request.

**Quality control:**
Glycerol-Free HS Tth DNA Polymerase activity is assayed by measuring amplification of different sized fragments using both RT-qPCR and qPCR, in comparison to a reference enzyme. The Tth DNA polymerase and its components are extensively tested for activity, processivity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

**Notes:**
For research and further manufacturing use only.

## Description

Glycerol-Free HS Tth DNA Polymerase is a thermostable DNA-dependent DNA polymerase from Thermus thermophilus with high reverse transcriptase activity in the presence of $Mn^{2+}$ ions. Its high processivity and reverse transcriptase activity at elevated temperatures allow Glycerol-Free HS Tth DNA Polymerase to overcome the problems posed by RNA secondary structures. Glycerol-Free HS Tth DNA Polymerase uses antibody-mediated hot start technology that enhances the specificity and sensitivity of PCR. It is supplied with a 5x Reaction Buffer that contains dNTPs and excipients required for lyophilization. Glycerol-Free HS Tth DNA Polymerase enables flexible and scalable reaction volumes, and is suitable for applications such as fast RT-qPCR amplification of difficult templates from crude samples.

## Kit components

**Table 1**

| Component |
|---|
| Glycerol-Free HS Tth DNA Polymerase, 200 U/µL |
| Lyo-Ready™ Tth Reaction Buffer, 5x |
| GF Enzyme Dilution Buffer, 10x |

## Users Guidelines

1. Prepare a working enzyme dilution of Glycerol-Free HS Tth DNA Polymerase to use in reaction. (see composition in table 2).

**Table 2**

| Reagent | Volume | Working Concentration |
|---|---|---|
| Glycerol-Free HS Tth DNA Polymerase, 200 U/µL | 1.5 µL | 5 U/µL |
| GF Enzyme Dilution Buffer, 10x | 6 µL | 1x |
| Water | To 60 µL | - |

### Master Mix preparation

1. Assemble the reaction in a microcentrifuge tube on ice as indicated in table 3. The volumes shown are for a 20 µL reaction.
2. Vortex thoroughly and pulse-spin in a microcentrifuge.

**Table 3**

| Reagent | Volume | Final Reaction concentration |
|---|---|---|
| Glycerol-Free HS Tth DNA Polymerase, 5 U/µL | 2 µL | 0.5 U/µL |
| Lyo-Ready™ Tth Reaction Buffe, 5x | 4 µL | 1x |
| Primer-Probe Mix, 20x* | 1 µL | 1x |
| 100 mM $MgCl_2$ (for DNA templates) or 100 mM $Mn(OAC)_2$ (for RNA/DNA templates)** | 1.2 µL | 5.8 mM |
| Template | X µL | As required |
| Water | Up to 20 µL | |

**Primer and probe concentration needs to be optimised*

*** Please note that this kit does not contain $MgCl_2$ or $Mn(OAC)_2$ solutions. The concentration required with this mix has been optimised to be 5.8 mM in final reaction, however costumers are advised to optimise the concentration of $MgCl_2$ or $Mn(OAC)_2$ for their individual assay needs*

## Lyophilization Protocol

1. Assemble the reaction in a microcentrifuge tube on ice as indicated in table 4. The volumes shown are for a 20 µL reaction.
2. Vortex thoroughly and pulse-spin in a microcentrifuge.
3. Refer to the «MDX205 Lyophilization and Post-Lyophilization User Guideline» for recommended cycling conditions for lyophilization.
4. Seal and store lyophilized material at room temperature until ready for use.
5. Rehydrate the lyophilized master mix in the reaction vials with 20 µL solution containing the template and 5.8 mM $MgCl_2$ (for DNA templates) or $Mn(OAC)_2$ (for RNA and DNA templates).
6. Vortex thoroughly and pulse-spin in a microcentrifuge.
7. Start qPCR reaction following cycling conditions shown below.

**Table 4**

| Reagent | Volume |
|---|---|
| Glycerol-Free HS Tth DNA Polymerase, 200 U/µL | 2 µL |
| Lyo-Ready™ Tth Reaction Buffer, 5x | 4 µL |
| Primer-Probe Mix, 20x | 1 µL |
| Water | Up to 15 µL |

## Assay setup

The qPCR conditions in Table 5 are suitable for amplicons of up to 200 bp. These cycling parameters have been optimized for on a number of platforms, however they can be varied to suit different machine-specific protocols.

**Table 5**

| Step | Temperature | Time | Cycles |
|---|---|---|---|
| Polymerase activation | 95 °C | 2 min | 1 |
| Reverse Transcription* | 50 to 65 °C | 5 to 10 min | 1 |
| Denaturation | 95 °C | 10 s | 45 |
| Annealing/Extension** | 60 °C | 25 s | |

** The reverse transcriptase step is required only for the RT-qPCR reaction that contain $Mn(OAC)_2$ . When multiplexing, the reverse transcription reaction time can be extended up to 20 minutes.*
*** The annealing/extension time can be extended up to 60 seconds and/or the annealing/extension temperature can be increased up to 65°C*

Meridian Life Science Inc.
USA

Tel: +1 901 382 8716
Fax: +1 901 382 0027

## Technical Support

For any technical enquiries, please contact our Technical Support team via email at: mbi.tech@meridianlifescience.com

# Glycerol-Free HS Tth DNA Polymerase

# User Guide

The guidelines in this document can help users avoid problems in lyophilization. For storage and stability, expiry and general handling of these product pre-lyophilization, please refer to the individual Product Handling Guides.

**Safety precautions:**

Read and understand the SDS (Safety Data Sheets) before handling the reagents. Copies of these SDSs are available on our website or upon request.

There are several advantages for lyophilization, including room temperature shipping and storage, extended shelf-life and increased flexibility in sample volume. In order to be compatible with lyophilization however, enzyme preparations must be glycerol-free and include specialized lyophilization-excipients that preserve the mixture as it is exposed to various lyophilization conditions including freezing, temperature ramps, vacuum and dehydration. An ideal lyophilization formulation should stabilize an enzyme in a freeze-dried format and allow very fast rehydration and reactivation of the enzyme preparations, without impacting its performance post rehydration.

## Lyophilization

- The lyophilization cycle protocol in table 1 is suitable for lyophilization of the Glycerol-Free HS Tth DNA Polymerase added to Lyo-Ready™ Tth Reaction Buffer, 5x in standard reaction tubes and plates. These parameters are provided as a guidance only and should be optimized to different user formats and systems.
- An annealing step can be added during the freezing step to assist crystallization of amorphous material.
- Combined primary and secondary drying time can be extended up to 24 hours.
- For product containing excipients, there should be no need to add any further excipients to assist lyophilization.

**Table 1.** Lyophilization guidelines

| Step | Temperature | Time | Description |
|---|---|---|---|
| Freezing | +4 °C | 10 min | Hold |
| | -45 °C | 1.0 °C/min | Ramp |
| Primary Drying | -45 °C | 180 min | Hold |
| | -40 °C | 0.5 °C/min | Ramp |
| | -40 °C | 720 min | Hold |
| Secondary Drying | +25 °C | 0.5 °C/min | Ramp |
| | +25 °C | 240 min | Hold |

## Post-Lyophilization

For maximum shelf-life, we suggest packaging lyophilized material under inert gas conditions (e.g. nitrogen or argon) and insert a desiccant sachet to improve stability. Pouches should be heat-sealed and labelled.