

# Product Information

## Cheetah™ FLEX HotStart Taq

### Unit Size (Enzyme Only):

29079-1ML: 1 mL, 5 kU  
29079-10ML: 10 mL, 50 kU  
29079-100ML: 100 mL, 500 kU

### Kit Contents: Cheetah™ FLEX HotStart Taq (500 U Taq with Buffer Pack)

| Component                                                             | Size           |
|-----------------------------------------------------------------------|----------------|
| 29079-100UL: Cheetah™ FLEX HotStart Taq, 5 U/uL                       | 100 uL (500 U) |
| 29050B: Cheetah™ Taq Dilution Buffer, 10X (without Mg <sup>2+</sup> ) | 1.5 mL         |
| 29050C: 25 mM MgCl <sub>2</sub>                                       | 1.5 mL         |

### Storage and Handling

Store at -20°C. Product is stable for at least 12 months from date of receipt when stored as recommended.

### Product Description

Cheetah™ FLEX HotStart Taq is Biotium's proprietary chemically modified Taq polymerase designed for reducing non-specific DNA amplification due to primer-dimer formation in PCR. Cheetah™ Taq also has a better shelf life than AmpliTaq Gold® due to its unique chemical modification, which is less likely to form intramolecular cross-links.

The activation time for Cheetah™ Taq is only about 2 minutes at 95°C, which is 5- to 10-times faster than that for AmpliTaq Gold® or HotStarTaq®, making it especially advantageous for fast-cycling qPCR. Furthermore, unlike AmpliTaq Gold®, activation of Cheetah™ Taq is relatively insensitive to pH, permitting the use of reaction buffers between pH 6 and pH 10. Unlike antibody-based hot-start Taq polymerases, chemically modified Cheetah™ Taq is free of animal DNA, and its activity is completely suppressed prior to heat activation. After heat activation, Cheetah™ Taq has the same functional properties and fidelity as native Taq polymerase.

In addition to its applications in qPCR, Cheetah™ FLEX HotStart Taq has been validated by third parties in qPCR-based proximity extension assay (PEA) for protein detection.

Cheetah™ FLEX HotStart Taq is available as a ready-to-use kit, which includes a buffer pack, and as a standalone enzyme for assay development and bulk purchase. Our Forget-Me-Not™ qPCR Master Mixes featuring Cheetah™ Taq (see Related Products) may also be of interest.

We also offer Cheetah™ PEA-PLEX HotStart Taq, which is a specialized formulation of Cheetah™ Taq validated by third parties for NGS-based high-throughput PEA.

### PCR Reaction Setup

The following reaction setup is provided as a general starting point for optimization:

| Reaction Component            | Volume for 50 uL Reaction | Final Concentration | Range for Optimization |
|-------------------------------|---------------------------|---------------------|------------------------|
| Molecular Biology Grade Water | To 50 uL total            | N/A                 | N/A                    |
| 10X Cheetah Dilution Buffer   | 5 uL                      | 1X                  | N/A                    |
| 25 mM MgCl <sub>2</sub>       | 5 uL                      | 2.5 mM              | 1.5-3.5 mM             |
| 10 mM dNTPs                   | 1 uL                      | 0.2 mM each dNTP    | N/A                    |
| 10 uM forward primer          | 1 uL                      | 0.2 uM              | 0.1-1 uM               |
| 10 uM reverse primer          | 1 uL                      | 0.2 uM              | 0.1-1 uM               |
| Cheetah™ FLEX Taq             | 1 uL                      | 0.1 U/uL            | 0.02-0.1 U/uL          |
| Template DNA                  | x uL                      | <1 ug total         | N/A                    |

### Cycling Protocols

Choice of cycling protocol depends on your instrument capability and on the nature of your amplicon. If your instrument does not support fast cycling, use the parameters recommended in your instrument manual.

#### 1. Two-step fast cycling protocol

This protocol should be applicable to most amplifications where the primer melting temperature (T<sub>m</sub>) is designed to be 60°C.

| Cycling Step         | Temperature | Hold Time       | Number of Cycles |
|----------------------|-------------|-----------------|------------------|
| Enzyme Activation    | 95°C        | 2 minutes       | 1                |
| Denaturation         | 95°C        | 1-15 seconds    | 25-35            |
| Annealing/ Extension | 60°C        | 1 minute per kb |                  |

## 2. Three-step fast cycling protocol

Use this protocol when optimal primer annealing and extension temperatures are desired.

| Cycling Step      | Temperature                     | Hold Time       | Number of Cycles |
|-------------------|---------------------------------|-----------------|------------------|
| Enzyme Activation | 95°C                            | 2 minutes       | 1                |
| Denaturation      | 95°C                            | 1-15 seconds    | 25-35            |
| Annealing         | 5°C below primer T <sub>m</sub> | 5-30 seconds    |                  |
| Extension         | 72°C                            | 1 minute per kb |                  |
| Final Extension   | 72°C                            | 5 minutes       | 1                |

### Analysis

Analyze 10 µL of the PCR reaction by agarose gel electrophoresis to confirm successful amplification of the desired amplicon. PCR reactions may be stored at -20°C for downstream applications.

## Related Products

| Cat. No.    | Product                                                       |
|-------------|---------------------------------------------------------------|
| 29138       | Cheetah™ PEA-PLEX HotStart Taq (5 U/µL, Enzyme Only)          |
| 31000       | EvaGreen® Dye 20X in Water                                    |
| 31077       | EvaGreen® Plus Dye 20X in Water                               |
| 31079       | EvaRuby™ Dye 20X in Water                                     |
| 29052       | ROX Passive Reference Dye                                     |
| 29087       | VeriFluor™ Far-Red Passive Reference Dye                      |
| 31041-31042 | Forget-Me-Not™ EvaGreen® qPCR Master Mix (Separate ROX)       |
| 31043-31044 | Forget-Me-Not™ Universal Probe Master Mix (Separate ROX)      |
| 31078       | N-Flux™ 5X Digital PCR Master Mix                             |
| 40054       | dNTP Mix, 10 mM each                                          |
| 41024       | Water, Ultrapure Molecular Biology Grade                      |
| 29051       | EvaEZ™ Fluorometric DNA Polymerase Activity Assay             |
| 41041-41042 | Precast GelRed® Agarose Gels, 1% Agarose/TAE                  |
| 41043       | EMBER™ Ultra DNA Gel Kit                                      |
| 41044       | EMBER™ Ultra RNA Gel Kit                                      |
| 41011       | GelRed® Prestain Plus 6X DNA Loading Dye                      |
| 41003       | GelRed® 10,000X in Water                                      |
| 41005       | GelGreen® 10,000X in Water                                    |
| 41028       | Agarose LE, Ultra-Pure Molecular Biology Grade                |
| 41029       | GelRed® Agarose LE                                            |
| 41030       | GelGreen® Agarose LE                                          |
| 22031       | 1X TAE (1L) Buffer Powder Packets                             |
| 22032       | 1X TBE (1L) Buffer Powder Packets                             |
| 41039       | Go-Go™ Fast DNA Gel Running Buffer, 50X                       |
| 31028       | AccuClear® Ultra High Sensitivity dsDNA Quantitation Kit      |
| 31066       | AccuGreen™ High Sensitivity dsDNA Quantitation Kit for Qubit® |
| 31080       | 1 kb DNA Ladder in TE buffer                                  |
| 31081       | 100 bp DNA Ladder in TE buffer                                |
| 31084       | 1 kb DNA Ladder, Ready-to-Load                                |
| 31085       | 100 bp DNA Ladder, Ready-to-Load                              |
| E90005      | Gel-Bright™ Laser Diode Gel Illuminator                       |

Please visit our website at [www.biotium.com](http://www.biotium.com) for information on our life science research products, including environmentally friendly EvaGreen® qPCR master mixes, Cheetah™ PEA-PLEX HotStart Taq, and Forget-Me-Not™ qPCR Master Mixes featuring Cheetah™ Taq.

AmpliTaq Gold is a registered trademark of Thermo Fisher Scientific; HotStarTaq is a registered trademark of Qiagen.

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