Technical Bulletin

Glycerol-Free JumpStart™ Taq DNA Polymerase

Lyophilization-compatible

D9310

Storage Temperature 2–8 °C

Product Description

Glycerol-Free JumpStart™ *Taq* DNA Polymerase is a lyophilization-compatible blend of *Taq* DNA Polymerase and JumpStart™ *Taq* antibody. The glycerol-free formulation allows the user to lyophilize PCR master mixes, which can be stored at ambient temperature and used up to one year later without reagent degradation.

Taq DNA polymerase is inactivated at ambient temperature through formulation with neutralizing JumpStart™ *Taq* antibody, allowing for a simple, efficient procedure through hot-start PCR[1]. Raising the reaction temperature above 70 °C in the initial PCR denaturation step leads to complex dissociation, resulting in fully active polymerase.

Antibody-mediated hot-start PCR can significantly improve the results of DNA amplification by reducing the generation of nonspecific products and primer-dimer artifacts.

Typical applications for Glycerol-Free JumpStart™ *Taq* include lyophilization of PCR master mixes and any other DNA amplification protocol in which glycerol is contraindicated, such as freeze-drying and automated PCR applications.

The enzyme is supplied at 5 units/µL in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.05 mM EDTA, 5 mM DTT, and stabilizers, without $MgCl_2$.

Unit Definition

One unit incorporates 10 nmol of total deoxyribonucleoside triphosphates into acid precipitable DNA in 30 minutes at 74 °C.

Materials and Reagents Required

(But not included)

- 10x PCR Buffer without $MgCl_2$ (Cat. No. P2317)
- Deoxynucleotide (dNTP) Mix, containing 10 mM of each dATP, dCTP, dGTP, and dTTP sodium salts (Cat. No. D7295)
- Nuclease-free water (Cat. No. W4502)
- Magnesium chloride solution, 25 mM (Cat. No. M8787)
- PCR tubes or plates
- Primers specific to gene target
- Template DNA
- Thermal cycler

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2–8 °C. In the supplied storage buffer and at the supplied concentration, Glycerol-Free JumpStart™ *Taq* is stable for 2 years if the solution is handled aseptically. Freezing Glycerol-Free JumpStart™ *Taq* is not recommended and may adversely affect its functionality.

Procedure for Use

Preparation of a PCR Mix

To perform routine PCR, assemble reaction mixtures with the contents listed in Table 1. Preparation of a master mix containing all components except for template is recommended for best assay reproducibility.

Table 1. PCR Composition

Reagent	Final Concentration	Amount per 20 µL reaction
10X PCR buffer	1X	2 µL
dNTP Mix, 10 mM	200 µM	0.4 µL
$MgCl_2$	See Table 2	Variable
Glycerol-Free JumpStart™ *Taq*	0.05 U/µL	0.2 µL
Primers	Variable	Variable
Template	Variable	Variable
Nuclease-free Water	-	To 20 µL

Because the Glycerol-Free JumpStart™ *Taq* is a magnesium ion-dependent enzyme, the optimal concentrations of template DNA, primers, and $MgCl_2$ will depend on the system being utilized. The optimal $MgCl_2$ concentration is also dependent upon the intended application. See Table 2 for recommended ranges of $MgCl_2$ to use in reactions containing Glycerol-Free JumpStart™ *Taq*.

Table 2. Recommended $MgCl_2$ concentrations by application

Application	Recommended $MgCl_2$ concentration range
Endpoint PCR	1.5 – 3.5 mM
SYBR green-based qPCR	3 – 5 mM
Probe-based qPCR	4 – 7 mM

Lyophilization of a PCR Mix

To lyophilize Glycerol-Free JumpStart™ *Taq* alone or in a PCR master mix format with or without primers, lyoprotectants must be added to stabilize the enzyme through the freeze-drying process. PCR-compatible lyoprotectants may include, but are not limited to, mannitol, trehalose, PEG, and bovine serum albumin[2]. Formulations must be optimized to meet individual assay needs.

Post-Lyophilization PCR

Resuspend lyophilized reagents in nuclease-free water. Add DNA template and primers (if not included in lyophilized mixture) and bring to the desired reaction volume. Mix gently and briefly centrifuge to collect the solution at the bottom of the tube.

PCR Amplification

Amplification parameters will vary depending on the primers and the instrument used. It may be necessary to optimize the system for individual primers, template, and thermal cycler. A suggested thermocycling protocol is shown in Table 3.

Table 3. Thermocycling protocol using Glycerol-Free JumpStart™ *Taq*

Initial denaturation		94 °C	2 min
30 cycles	Denaturation	94 °C	15 sec
	Annealing	60 °C or 5 °C below lowest primer T_M	30 sec
	Extension	72 °C	1 min/kb
Final extension		72 °C	1 min
Hold		4 °C	∞

Amplified DNA can be evaluated by any standard method, including agarose gel electrophoresis, fluorescent dye intercalation, and DNA sequencing.

Related Products

- D-Mannitol (Cat. No. M4125)
- D-(+)-Trehalose dihydrate (Cat. No. T9531)
- PEG 20,000 (Cat. No. 81300)
- Bovine Serum Albumin (Cat. No. B8894 or B8667)
- 10X PCR buffer (Cat. No. P2192)
- Magnesium chloride solution, 1 M (Cat. No. M1028)
- JumpStart™ *Taq* DNA Polymerase (Cat. No. D9307 or D4184)

References

1. Dieffenbach, C., and Dveksler, G., (Eds),. *PCR Primer: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2003.
2. Xu, Jiasu *et al*. Room-temperature-storable PCR mixes for SARS-CoV-2 detection. Clinical biochemistry vol. 84:73-78 (2020).

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D9310dat Rev 09/21