T4 Gene 32 Protein (10 mg/mL)

Product Description

T4 Gene 32 Protein (gp32) is a specific single-stranded DNA (ssDNA) binding protein that is involved in T4 bacteriophage replication and repair.[1] gp32 binds and stabilizes ssDNA and is utilized as an additive in molecular biology assays to increase DNA yield.[2]

T4 Gene 32 Protein is available in glycerol-containing and glycerol-free formats. Glycerol-free formats are suitable for downstream lyophilization.

Relevant Applications*

- PCR[2]
- Electron microscopy[3]
- Restriction enzyme digests[4]
- RT-PCR[5]
- Whole genome amplification (WGA)[6]
- Helicase-dependent amplification[7]
- Recombinase Polymerase Amplification (RPA)[8] (see **Reaction Setup for Recombinase Polymerase Amplification (RPA) of DNA**)

*Watchmaker Genomics has not tested or validated T4 Gene 32 Protein in all applications listed.

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Kit Contents

Kit code	Description	Component volume		
		25 µL	50 µL	500 µL
7K0071-50UL **7K0071-500UL**	T4 Gene 32 Protein (10 mg/mL)	–	500 µg	5000 µg
7K0127-25UL **7K0127-500UL**	T4 Gene 32 Protein – Glycerol-free (10 mg/mL)	250 µg	–	5000 µg

For custom formats, contact the **Sales Team** at sales@watchmakergenomics.com.

Functionality

- T4 Gene 32 Protein is supplied at a concentration of 10 mg/mL. The ssDNA binding affinity of gp32 (reported as K_d) is determined by incubating a dilution series of gp32 with a constant amount of fluorescently labeled ssDNA. The resulting signal change in fluorescence polarization is used to determine the binding affinity (K_d).
- T4 Gene 32 Protein (glycerol-containing) storage buffer: 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl, 50% Glycerol.
- T4 Gene 32 Protein – Glycerol-free storage buffer: 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 100 mM NaCl.

Storage and Handling

- Glycerol-containing kits are shipped on ice packs. Upon receipt, store at -20°C ±5°C.
- Glycerol-free kits are shipped on dry ice. Upon receipt, store at -80°C ±10°C.

Keep all components and reaction mixes on ice or a cooled reagent block during routine use. Take care to mix solutions gently but thoroughly before use and during reaction setup.

When stored and handled at recommended temperatures, the product will retain full performance until the expiry date printed on the kit box.

Heat Inactivation

65°C for 20 minutes

Reaction Setup for Recombinase Polymerase Amplification (RPA) of DNA

T4 Gene 32 Protein can be used for Recombinase Polymerase Amplification (RPA) of DNA when combined with glycerol-free Watchmaker Genomics reagents, T4 UvsX DNA Recombinase (7K0124), T4 UvsY Protein (7K0126), and *Bsu* DNA Polymerase, Large Fragment (7K0128). We suggest assembling the reaction components for RPA in 3 separate steps prior to incubation in a thermocycler or heating block:

1. RPA Enzyme Mix (10X) preparation
2. Reaction Buffer (2X) preparation with primers
3. Combination of RPA Enzyme Mix with Reaction Buffer to 1X final concentration and addition of DNA template and $Mg(CH_3CO_2)$

Note: All preparation steps must be carried out on ice.

1. RPA Enzyme Mix (10X) preparation:

A starting ratio at which to combine *Bsu* DNA Polymerase, Large Fragment, TT4 UvsX DNA Recombinase, T4 UvsY Protein, and T4 Gene 32 Protein to make a 10X Enzyme Mix is suggested below. We suggest adding creatine kinase (not supplied) to this 10X Reaction Mix. Optimization of this ratio may be required. The 10X Enzyme Mix should be assembled on ice and used immediately.

RPA Enzyme Mix (10X):

Component	Supplier	Final Concentration (10X)
Bsu DNA Polymerase, Large Fragment – Glycerol-free (7K0128)	Watchmaker Genomics	5 U/µL
T4 UvsX DNA Recombinase – Glycerol-free (7K0124)	Watchmaker Genomics	0.6 µg/µL
T4 UvsY Protein – Glycerol-free (7K0126)	Watchmaker Genomics	0.3 µg/µL
T4 Gene 32 Protein – Glycerol-free (7K0127)	Watchmaker Genomics	0.9 µg/µL
Creatine Kinase	Not available from Watchmaker Genomics (source separately)	Variable (20 to 100 ng/uL)

2. Reaction Buffer (2X) preparation with primers:

Reaction conditions for RPA can vary depending on the target DNA sequence or size. The following suggested 2X Reaction Buffer (reagents and primers not supplied by Watchmaker Genomics) can be used to test the initial RPA reaction. Further optimization may be required for optimal results. This Reaction Buffer (2X) should be assembled on ice:

- RPA Reaction Buffer (2X): 8 mM DTT, 10% PEG 20000, 100 mM Tris-acetate, pH 8.0, 200 mM KCH_3CO_2, 0.4 mM dNTPs, 6 mM ATP, 2% Trehalose, 20 mM Phosphocreatine, DNA target-specific primers. This RPA Reaction Buffer (2X) is stable for ~6 months if stored at -20°C.
- Primer Concentration (see **RPA Primer Design Guidelines**):
  - For single-plexed reactions, use an optimal primer concentration range between 0.3 – 0.6 µM in the final reaction volume.
  - For multiplexed reactions, use a target primer concentration range between 0.1 – 0.3 µM in the final reaction volume.

3. Combination of RPA Enzyme Mix with Reaction Buffer, template DNA and $Mg(CH_3CO_2)$

To minimize non-specific polymerization, the DNA sample and $Mg(CH_3CO_2)$ should be added last into the reaction tube. The final RPA reaction components should be assembled on ice in the following order:

Component	Final Concentration
Enzyme Mix (10X)	1X
RPA Reaction Buffer (2X) (including primers)	1X
DNA sample	Variable
10 mM $Mg(CH_3CO_2)$†	Variable

†Not supplied by Watchmaker Genomics.

The assembled reaction should be loaded into a thermocycler or heating block and incubation initiated immediately post-assembly.

Optimal RPA reaction temperatures range from 37 – 42°C with amplification time between 20 to 40 minutes.

The above reaction setup is suggested as a starting point for a RPA reaction. However, there are many factors which can be adjusted to optimize the reaction for maximum sensitivity, specificity and speed. Please contact support@watchmakergenomics.com for further technical support.

RPA Primer Design Guidelines

The following characteristics are of critical importance when designing RPA primers:

- The optimal length for RPA primers is 30 – 35 nt. Primers less than 30 nt are not recommended.
- Primer sequences should have between 30 – 70% GC content and no single or dinucleotide base repeats.
- Primer sequences should also be devoid of complementary sequences which promote secondary structure hairpin loops and prevent self-dimerization or primer-primer interactions.
- Amplicon sequences should have between 35 – 60% GC content, with an optimal length of 150 – 450 bp.
- If possible, the primer should end with a G or C on the 3' terminus.

References

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8. Lobato IM, O'Sullivan CK. Recombinase polymerase amplification: Basics, applications and recent advances. *TrAC Trends in Analytical Chemistry*. 2018; 98:19 – 35. doi.org/10.1016/j.trac.2017.10.015

Revision History

Version	Description	Date
1.0	• First protocol release	1/2024
2.0	• Inclusion of new product details for T4 Gene 32 Protein Glycerol-free	6/2025
2.1	• Addition of guidelines for reaction setup for RPA of DNA	7/2025

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