

## Bsu DNA Polymerase, Large Fragment (50 U/μL)

### Product Description

*Bsu* DNA Polymerase, Large Fragment is a strand-displacing polymerase which retains the 5'→3' polymerase activity of the *Bacillus subtilis* DNA polymerase, but lacks the 5'→3' exonuclease domain. This large fragment lacks 3'→5' exonuclease activity.

The Watchmaker *Bsu* DNA Polymerase, Large Fragment is available in glycerol-free and glycerol-containing formats.

### Relevant Applications\*

- Recombinase Polymerase Amplification (RPA)<sup>1</sup> (see **Reaction Setup for Recombinase Polymerase Amplification (RPA) of DNA**)
- Isothermal amplification
- DNA strand displacement synthesis
- Second strand cDNA synthesis
- Random primer labelling

\*Watchmaker Genomics has not tested or validated *Bsu* DNA Polymerase, Large Fragment in all applications listed.

### Table of Contents

<b>Product Description</b> .....	1
<b>Relevant Applications</b> .....	1
<b>Unit Definition and Reaction Buffer Composition</b> .....	1
<b>Kit Contents</b> .....	1
<b>Reaction Temperature</b> .....	2
<b>Storage and Handling</b> .....	2
<b>Heat Inactivation</b> .....	2
<b>Reaction Setup for Recombinase Polymerase Amplification (RPA) of DNA</b> .....	2
<b>RPA Primer Design Guidelines</b> .....	3
<b>References</b> .....	3
<b>Revision History</b> .....	3

### Unit Definition and Reaction Buffer Composition

- 1U of *Bsu* DNA Polymerase, Large Fragment is defined as the amount of enzyme required to incorporate 200 nM dNTPs in 60 minutes at 37°C.
- Recommended *Bsu* DNA Polymerase, Large Fragment Reaction Buffer 10X (not provided with kit): 500 mM NaCl, 100 mM Tris-HCl, pH 7.9, 100 mM MgCl<sub>2</sub>, 10 mM DTT. This buffer can be used for strand-displacement amplification. For RPA of DNA applications, refer to **Reaction Setup for Recombinase Polymerase Amplification (RPA) of DNA**.

### Kit Contents

Kit code	Description	kU amount	Component volume (μL)
<b>7K0128-25UL</b>	<i>Bsu</i> DNA Polymerase, Large Fragment (50 U/μL), Glycerol-free	1.25	25
<b>7K0128-500UL</b>	<i>Bsu</i> DNA Polymerase, Large Fragment (50 U/μL), Glycerol-free	25	500
<b>7K0129-25UL</b>	<i>Bsu</i> DNA Polymerase, Large Fragment (50 U/μL)	1.25	25
<b>7K0129-500UL</b>	<i>Bsu</i> DNA Polymerase, Large Fragment (50 U/μL)	25	500

For larger volumes, higher concentrations, and custom formats, contact the **Sales Team** at [sales@watchmakergenomics.com](mailto:sales@watchmakergenomics.com).

## Reaction Temperature

*Bsu* DNA Polymerase, Large Fragment is active between 25°C to 42°C. The optimal polymerization temperature is 37°C.

## Storage and Handling

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

For non-RPA applications, keep all components and reaction mixes on ice or a cooled reagent block. For RPA reactions, bring *Bsu* DNA Polymerase to 25°C prior to use. Pipette-mix or pulse-vortex (2X) the enzyme before use. When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

## Heat Inactivation

75°C for 20 minutes

## Reaction Setup for Recombinase Polymerase Amplification (RPA) of DNA

*Bsu* DNA Polymerase, Large Fragment 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 T4 Gene 32 Protein (7K0127). 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<sub>3</sub>CO<sub>2</sub>)

### 1. RPA Enzyme Mix (10X) preparation:

All enzymes should be thawed and brought to 25°C prior to use. A starting ratio at which to combine *Bsu* DNA Polymerase, Large Fragment, T4 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 at 25°C and used immediately.

#### RPA Enzyme Mix (10X):

Component	Supplier	Final Concentration (10X)
<i>Bsu</i> 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/μL)

### 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) may be assembled at 25°C.

- RPA Reaction Buffer (2X): 8 mM DTT, 10% PEG 20000, 100 mM Tris-acetate, pH 8.0, 200 mM KCH<sub>3</sub>CO<sub>2</sub>, 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<sub>3</sub>CO<sub>2</sub>)

To minimize non-specific polymerization, the DNA sample and Mg(CH<sub>3</sub>CO<sub>2</sub>) should be added last into the reaction tube. The final RPA reaction components should be assembled at 25°C 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 <sub>3</sub> CO <sub>2</sub> ) <sup>†</sup>	Variable

<sup>†</sup>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](mailto:support@watchmakergenomics.com) for further technical support.

## Revision History

Version	Description	Date
1.0	• First protocol release	11/2025
1.1	• Thawing conditions for Bsu DNA Polymerase revised • Temperature for RPA Enzyme Mix preparation and RPA reaction component assembly changed	03/2026



5744 Central Avenue, Suite 100  
Boulder, CO 80301

[www.watchmakergenomics.com](http://www.watchmakergenomics.com)

For Research Use Only. Not for use in diagnostic procedures.

This content is covered by patents, trademarks, and/or copyrights owned or controlled by Watchmaker Genomics Inc.

For more information, please visit [watchmakergenomics.com/licenses](http://watchmakergenomics.com/licenses). The use of these products may require you to obtain additional third party intellectual property rights for certain applications.

© 2026 Watchmaker Genomics. All rights reserved.

All trademarks are the property of Watchmaker Genomics or their respective owners.

## 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

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

For Technical Support, please contact [support@watchmakergenomics.com](mailto:support@watchmakergenomics.com).