

## T4 UvsX DNA Recombinase – Glycerol-free (5 mg/mL)

### Product Description

T4 UvsX DNA Recombinase is an enzyme derived from bacteriophage T4 that is analogous to the bacterial RecA protein. T4 UvsX DNA Recombinase serves an important role in homologous recombination, and in the case of isothermal amplification methods, the enzyme facilitates the priming of the amplification site when utilized in the presence of a DNA template and primer oligos. This enzyme, in combination with the T4 UvsY Protein and T4 Gene 32 Protein, can be used to form recombination complexes which then allow *Bsu* DNA Polymerase to initiate polymerization via the recombinase polymerase amplification (RPA) method.

T4 UvsX DNA Recombinase is only available in glycerol-free format, which is suitable for downstream lyophilization.

### Relevant Applications

- Recombinase Polymerase Amplification (RPA)<sup>1,2,3</sup> (see **Reaction Setup for Recombinase Polymerase Amplification (RPA) of DNA**)

### Kit Contents

| Kit code     | Description                              | Component volume |         |
|--------------|--|------------------|---------|
|              |  | 25 µL            | 500 µL  |
| 7K0124-25UL  | T4 UvsX Enzyme – Glycerol-free (5 mg/mL) | 125 µg           | 2500 µg |
| 7K0124-500UL |  |                  |         |

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

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### Storage and Handling

T4 UvsX DNA Recombinase – Glycerol-free is shipped on dry ice. Upon receipt, store at -80°C ±10°C. Thaw and bring to 25°C with intermittent pipette-mixing or pulse-vortexing prior to use. When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

### Heat Inactivation

70°C for 20 minutes

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

T4 UvsX DNA Recombinase can be used for Recombinase Polymerase Amplification (RPA) of DNA when combined with glycerol-free Watchmaker Genomics reagents, T4 UvsY Protein (7K0126), T4 Gene 32 Protein (7K0127) 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<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/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) 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.

### 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>
2. Li J, Macdonald J, von Stetten F. Review: a comprehensive summary of a decade development of the recombinase polymerase amplification. *Analyst*. 2019; 144(1):31 – 67. <https://doi.org/10.1039/C8AN01621F>
3. Piepenburg O, Williams CH, Stemple DL, Armes NA. DNA detection using recombination proteins. *PLoS Biol*. 2006; 4(7):e204. <https://doi.org/10.1371/journal.pbio.0040204>

### Revision History

| Version | Description  | Date    |
|---------|--|---------|
| 1.0     | • First protocol release   | 07/2025 |
| 1.1     | • Thawing conditions for T4 UvsX DNA Recombinase revised<br>• Temperature for RPA Enzyme Mix preparation and RPA reaction component assembly changed | 03/2026 |



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