Glycerol-Free T4 UvsX DNA Recombinase

Manual

Catalog #	3562GF	3565GF	3567GF
Package Size	100µg	500µg	1000µg
Volume	20µl	100µl	200µl
Concentration	5µg/µl		

Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Intact Genomics, Inc.

Table of Contents

Description:

Homologous recombination is important for the error-free repair of DNA double-strand breaks and for replication fork restart. Recombinases of the RecA/RAD51 family perform the central catalytic role in this process. UvsX recombinase is the RecA/Rad51 ortholog of bacteriophage T4. Intact Genomics (ig®) UvsX and other recombinases form presynaptic filaments on ssDNA that are activated to search for homology in dsDNA and to perform DNA strand exchange (1-3).

Protein Purity:

The physical purity of this enzyme is ≥98% as assessed by SDS-PAGE with Coomassie® blue staining (Fig. 1).

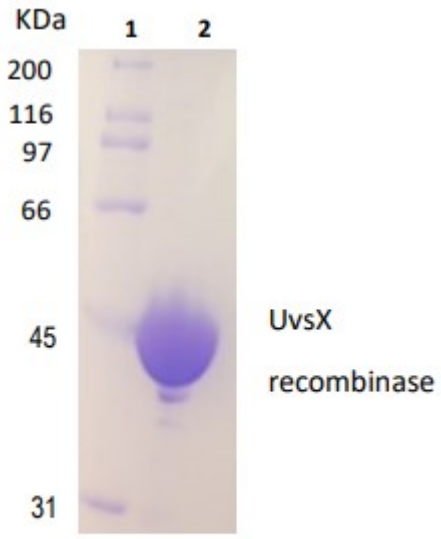


Fig. 1:
Lane 1, Protein marker
Lane 2, UvsX Recombinase

Specifications:

Product Source: *E. coli* BL21 (DE3) strain expressing T4 UvsX gene

Format: Tubes

Shipping condition: Dry ice

Product Components:

- Glycerol-Free T4 UvsX Recombinase
- 10x UvsX Recombinase Reaction Buffer
- 20 mM Tris-acetate pH 7.8, 100 mM Potassium acetate 10 mM Magnesium acetate, 1 mM DTT

Storage:

-20°C

Additional Buffer Information:

Suggested Storage/ Dilution Buffer:

50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA , pH 7.5 @ 25ºC

Quality Control:

UvsX recombinase is free from detectable nuclease activities .

Notes:

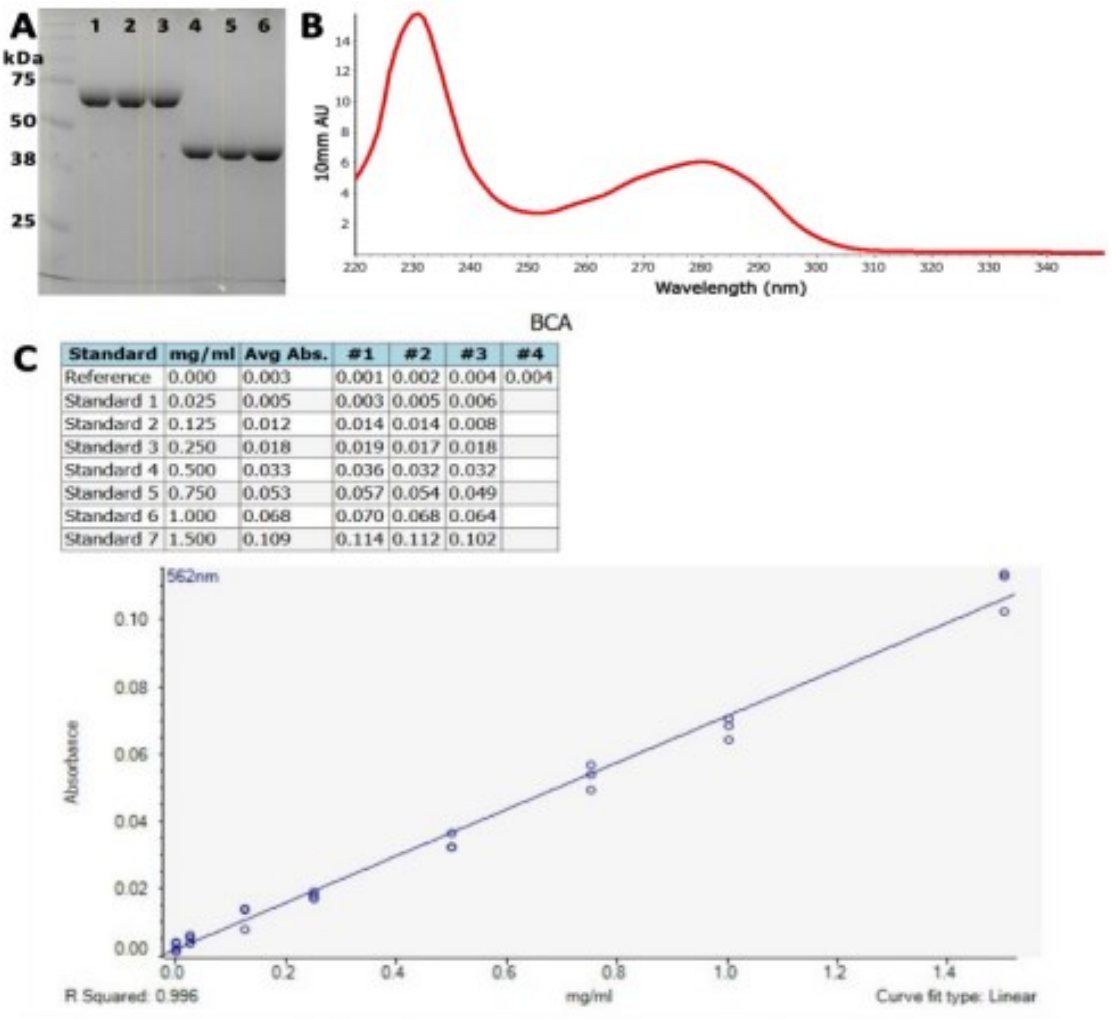
Glycerol acts as a cryoprotectant and protein stabilizer when added to the storage buffer of proteins and enzymes. However, in certain circumstances, it is preferred to omit glycerol from the buffer. This includes instances where the presence of glycerol may interfere, such as lyophilization, high-throughput instruments with sensitive fluidics or primary cell cultures. For our glycerol free products, it is recommended to use immediately upon thaw as without glycerol there will be significant activity loss with freeze/thaw cycles.

Enzyme Concentration:

IG uses orthogonal, 3-part approaches to determine the enzyme concentration to provide you with consistent and reliable enzymes for your needs. The quantity of a protein sample is assessed using densitometry with polyacrylamide gel electrophoresis (PAGE), UV absorbance spectra of native protein, and using a protein standard assay such as bicinchoninic acid assay (BCA) using bovine serum albumin (BSA) as a standard (Figure 1).

Why does IG use all three approaches?

1. Each method above has limitations. The limitations include experimental noise, accuracy, and susceptibility to buffer and/or enzyme conditions.
2. Each enzyme has unique physical properties that make a single approach to analyzing proteins a challenge. Each enzyme has a different protein sequence, different requirements to be stable in solution, and different requirements to retain its maximal activity. These differences can interfere with or convolute results, especially when compared to other enzymes. When used together, however, each method provides the scientist with independent measures of both enzyme and buffer purity and quality.



Standard	mg/ml	Avg Abs.	#1	#2	#3	#4
Reference	0.000	0.003	0.001	0.002	0.004	0.004
Standard 1	0.025	0.005	0.003	0.005	0.006	
Standard 2	0.125	0.012	0.014	0.014	0.008	
Standard 3	0.250	0.018	0.019	0.017	0.018	
Standard 4	0.500	0.033	0.036	0.032	0.032	
Standard 5	0.750	0.053	0.057	0.054	0.049	
Standard 6	1.000	0.068	0.070	0.068	0.064	
Standard 7	1.500	0.109	0.114	0.112	0.102	

Figure 1: Enzyme quantitation methods used by IG.

A) SDS-polyacrylamide gel electrophoresis. Ladder in 1st lane, 2 µg BSA (~67 kDa) as a standard in lanes 1-3, and IG enzymes (~40 kDa) in lanes 4-6. The yellow boxes are the areas evaluated by densitometry. The integrated band intensities of IG enzymes are compared with integrated band intensities from BSA to assay concentration.

B) UV spectrum of a clean IG enzyme with protein peaks at 230 nm and at 280 nm. An extinction coefficient at 280 nm is typically used to quantify protein using these spectra with buffer subtraction at 330 nm.

C) BCA standard curve for BSA. The curve is used to calculate an IG enzyme concentration using BSA as the standard.

Related Products:

- RPA Kit (Cat.#3526 & #3530)
- T4 gp32 Protein (Cat.# 3515)
- T4 UvsY Protein (Cat.# 3572)
- Bsu DNA Polymerase (Cat.# 3585)
- *Sau* DNA Polymerase (Cat.# 3595)
- Exonuclease III (Cat.# 3415)
- Exonuclease IV (Nfo) (Cat.# 3425)

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- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
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  Email: **sales@intactgenomics.com**
  Phone: (314) 942-3655 **|** Toll-free : 855-835-7172 **|** Fax: (314) 942-3656
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References:

1. Cromie GA, Connelly JC, Leach DR (2001) Recombination at double-strand breaks and DNA ends: conserved mechanisms from phage to humans. Mol Cell 8: 1163–1174

2. Michel B, Grompone G, Flores MJ, Bidnenko V (2004) Multiple pathways process stalled replication forks. Proc Natl Acad Sci U S A 101: 12783–12788

3. Liu J, Ehmsen KT, Heyer WD, Morrical SW (2011) Presynaptic filament dynamics in homologous recombination and DNA repair. Crit Rev Biochem Mol Biol 46: 240–270

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