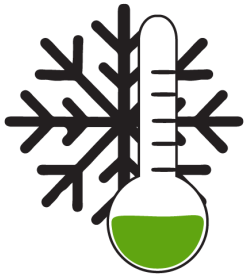
T4 UvsX DNA Recombinase

Manual



Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE

Catalog #	3562	3565	3576
Package Size	100 µg	500 µg	1000 µg

India Contact:
Life Technologies (India) Pvt. Ltd.
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Email: customerservice@lifetechindia.com
www.lifetechindia.com

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).

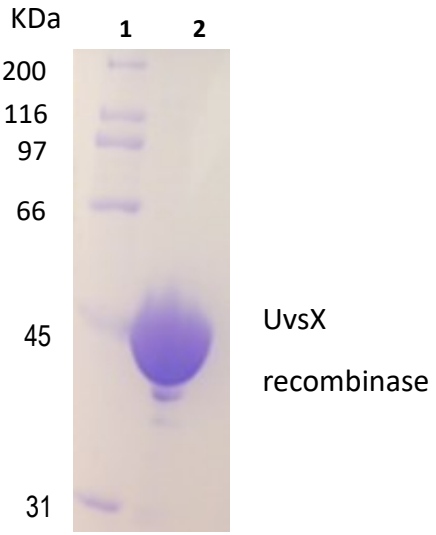


Fig. 1:
Lane 1, Protein marker
Lane 2, UvsX Recombinase

Product Source:

E. coli BL21 (DE3) strain expressing T4 UvsX gene.

Product Includes:

- UvsX Recombinase
- 10x UvsX Recombinase Reaction Buffer

1x UvsX Recombinase Reaction Buffer:

20 mM Tris-acetate pH 7.8, 100 mM Potassium acetate

10 mM Magnesium acetate, 1 mM DTT

Storage Buffer:

50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA

50% Glycerol, pH 7.5 @ 25ºC

Quality Control:

UvsX is free from detectable nuclease activities.

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

Related Products:

1. T4 gp32 Protein (Cat.# 3515)
2. T4 UvsY Protein (Cat.# 3572)
3. Bsu DNA Polymerase (Cat.# 3585)
4. Sau DNA Polymerase (Cat.# 3595)
5. Exonuclease III (Cat.# 3415)
6. Exonuclease IV (Nfo) (Cat.# 3425)

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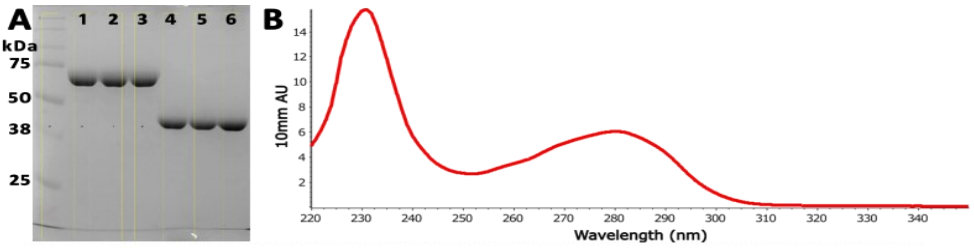
Enzyme Concentration:

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.



C

BCA

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	

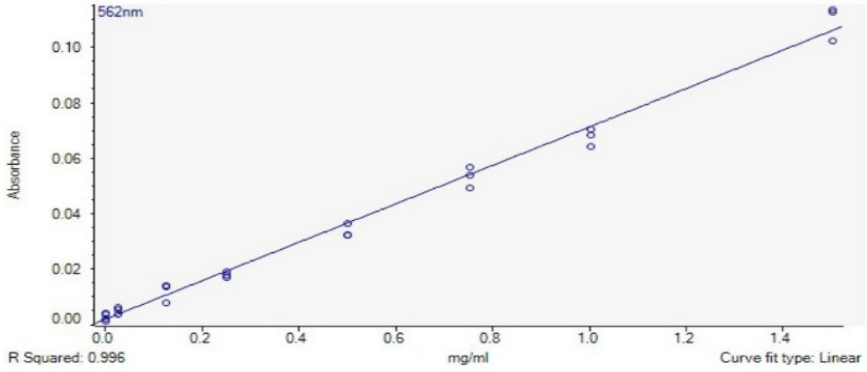


Figure 1: Enzyme quantitation methods used by IG. A) SDS-polyacrylamide gel electrophoresis. Ladder in 1st lane, 2 µg BSA (~67 kDa) as a standard in lanes 1-3, and IG enzymes (~40 kDa) in lanes 4-6. The yellow boxes are the areas evaluated by densitometry. The integrated band intensities of IG enzymes 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.

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India Contact:
Life Technologies (India) Pvt. Ltd.
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Email: customerservice@lifetechindia.com
www.lifetechindia.com