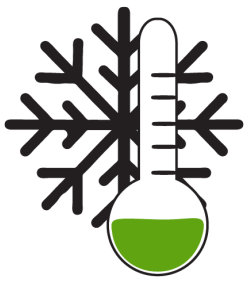
Glycerol-Free
T4 UvsY Protein

Manual



Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE

Catalog #	3572G	3575G	3577G
Package Size	100 µg	500 µg	1000µg

Intact Genomics, Inc.
www.intactgenomics.com

V2023 Rev 0

Glycerol-Free T4 UvsY Protein

Description:

Intact Genomics (ig®) UvsY is the phage T4 recombination mediator protein, and structural and biophysical studies provide insights into its role in T4 homologous recombination. During T4 homologous recombination, the UvsX recombinase must compete with the prebound gp32 single-stranded binding protein for DNA-binding sites and UvsY stimulates this filament nucleation event (1). UvsY is a 15.8-kDa protein with properties that are consistent with its role as mediator; it stimulates the DNA-dependent ATPase activity of UvsX, lowers the critical concentration of UvsX that is required for activity, and promotes strand exchange (2, 3). UvsY efficiently promotes the UvsX-catalyzed strand invasion reaction by recruiting to ssDNA–gp32 complexes, promotes the release of gp32, and favors the binding of UvsX.

Protein Purity:

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

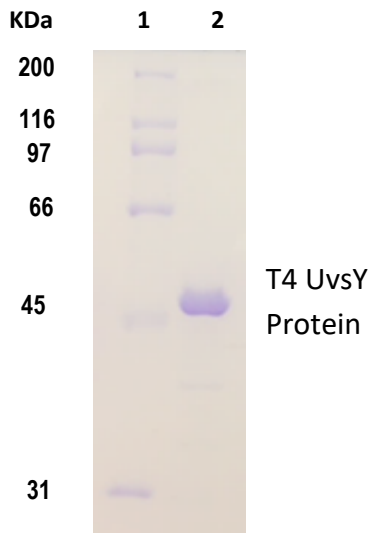


Fig. 1:
Lane 1, Protein marker
Lane 2, UvsY.

Product Source:

E. coli BL21 (DE3) strain expressing T4 UvsY gene with an N-terminal His tag.

This GST tag does not have any effect on UvsY activity.

Product Includes:

- Glycerol-Free T4 UvsY protein
- 10x UvsY Reaction Buffer

1x UvsY Reaction Buffer:

- 20 mM Tris-acetate, 100 mM Potassium acetate
- 10 mM Magnesium acetate, 1 mM DT
- pH 7.8 @ 25ºC

Storage Buffer:

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

Note:

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.

Quality Control:

UvsY is free from detectable nuclease activities.

References:

1. Beernink HT, Morrical SW. RMPs: Recombination/replication mediator proteins. Trends Biochem Sci. 1999;24(10):385–389.
2. Hashimoto K, Yonesaki T. The characterization of a complex of three bacteriophage T4 recombination proteins, uvsX protein, uvsY protein, and gene 32 protein, on single-stranded DNA. J Biol Chem. 1991;266 (8):4883–4888.
3. Beernink HT, Morrical SW. The uvsY recombination protein of bacteriophage T4 forms hexamers in the presence and absence of single-stranded DNA. Biochemistry. 1998;37(16):5673–5681

Related Products:

1. T4 gp32 Protein (Cat.# 3515)
2. T4 UvsX Protein (Cat.# 3562,)
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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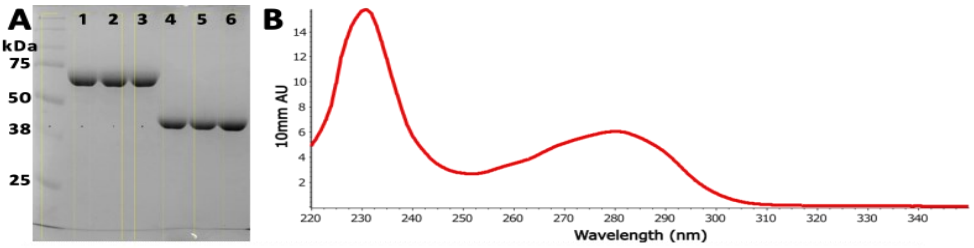
Enzyme Concentration:

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	

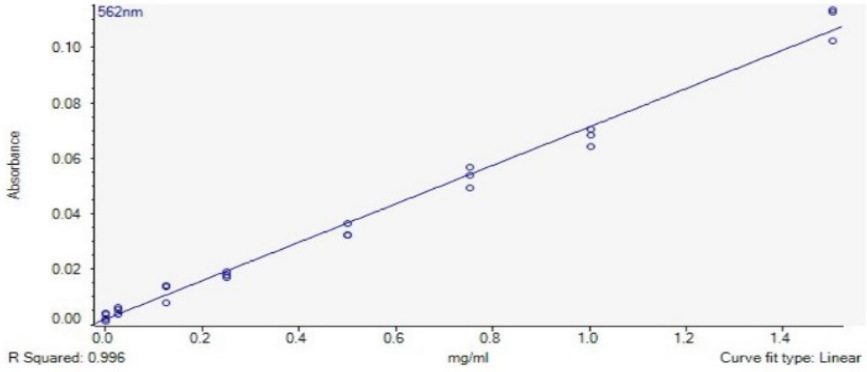


Figure 1: Enzyme quantitation methods used by IG. A) SDS-polyacrylamide gel electrophoresis. Ladder in 1st lane, 2 µg BSA (~67 kDa) as a standard in lanes 1-3, and IG enzymes (~40 kDa) in lanes 4-6. The yellow boxes are the areas evaluated by densitometry. The integrated band intensities of IG enzymes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

Intact Genomics, Inc.
11840 Westline Industrial Drive, Suite 120
St. Louis, MO. 63146

Phone: (314) 942-3655 | **Toll-free :** : 855-835-7172 | **Fax:** (314) 942-3656
Email: sales@intactgenomics.com

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