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Expression and Purification of recombinant Bst DNA polymerase (Bst)

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Diana A Tapia-Sidas

ABSTRACT

Bst is a type I DNA polymerase with strong strand displacement activity isolated from *Geobacillus stearothermophyllus* (previously *Bacillus steathermophylus*). Bst enzyme is a gold standard in isothermal nucleic acid amplification diagnostic techniques, especially in Loop-mediated isothermal amplification (LAMP). LAMP is a low-cost technique, provides a visual detection (when combined with pH indicators) and does not require the use of thermal cyclers. Also, Bst combined with thermostable reverse transcriptase can amplify RNA templates, in a technique known as RT-LAMP. RT-LAMP is useful for RNA virus and transcript detection and can be employed in circumstances that require mass production of diagnostic tests or limited availability of resources. This protocol shows the expression and purification procedure by FPLC of the Bst polymerase for its implementation in diagnostic techniques such as end-point colorimetric and real time fluorometric LAMP and RT-LAMP.

GUIDELINES

During the process of protein purification maintain all samples that contain the protein of interest in a cold environment to avoid protein degradation.

Protocol status: Working

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PROTOCOL integer ID: 63361

Keywords: Bacillus stearothermophylus, polymerase, Bst, protein expression, protein purification

MATERIALS

Reagents:

- Chaperone Plasmid Set **Takara Bio USA, Inc. Catalog #3340**
-
Chemically Competent E. coli One Shot™ BL21(DE3) **Invitrogen - Thermo Fisher Catalog #C600003**
- [M] 100 ng/µL pColdI-Bst plasmid Step 3.1
- Stock [M] 30 mg/mL chloramphenicol
- Stock [M] 100 mg/mL carbenicillin
- Stock [M] 1 Molarity (M) IPTG
- Stock [M] 10 % (v/v) Triton X-100
- 250 µL of SOC medium Step 1.4
- 8 mL of TFBI solution Step 2.4
- 2.5 mL of TFBII solution Step 2.5
- Cryotubes with 500 µL of 30% v/v glycerol (sterilized)
- LB agar plates
- Tubes with 3 mL Luria-Bertani (LB) medium
- Tubes with 5 mL Luria-Bertani (LB) medium
- Tubes with 5 mL Terrific Broth (TB) medium
- Flasks with 20 mL LB medium
- Flasks with 50 mL LB medium
- Flasks with 50 mL TB medium
- Flask with 100 mL LB medium
- Flasks with 1 L LB medium
- 250 mL lysis buffer A (LB-A) Step 5.5
- 250 mL Saline Buffer (SB) Step 9.2
- 500 mL Washing Buffer-AI (WB-AI) Step 10.2
- 500 mL Elution Buffer-AI (EB-AI) Step 10.5
- 1 L Desalting Buffer-A (DB-A) Step 11.2
- 500 mL Washing Buffer-AII (WB-AII) Step 12.2
- 500 mL Elution Buffer-AII (EB-AII) Step 12.5
- 500 mL Storage Buffer-A (SB-A) Step 13.1
- HisTrap HP 5mL **Cytiva Catalog #17524801**
- HiPrep 26/10 Desalting Column **Cytiva Catalog #17508701**
- HiTrap Heparin HP affinity column **Cytiva Catalog #17040701**
-
Quick Start™ Bradford 1x Dye Reagent **BioRad Sciences Catalog #5000205**
-

Precision Plus Protein™ Unstained Protein Standards **Bio-rad Laboratories Catalog #1610363**

- Tricine-SDS-PAGE electrophoresis solutions (Step 6.2)
- 8% polyacrylamide gels for Tricine-SDS-PAGE (Step 6.2)

Equipments:

- Thermomixer

Equipment	
Thermomixer® R	NAME
Dry block heating and cooling shaker, 120 V, 60 Hz, 1/cs	TYPE
Eppendorf	BRAND
T3317	SKU

- Orbital shaker

Equipment	
MaxQ™ HP Incubated Tabletop Orbital Shaker	NAME
MaxQ™ HP, 120 V 60 Hz, 6,5 A o 230 V 50/60 Hz, 3,2 A	TYPE
Thermo Scientific	BRAND
SHKE420HP	SKU
https://www.thermofisher.com/order/catalog/product/SHKE420HP	LINK

- Floor model orbital shaker

Equipment

MaxQ™ HP Incubated and Refrigerated Console Shakers	NAME
MaxQ™ 481 HP, 230 V, 50 Hz	TYPE
Thermo Scientific	BRAND
SHKE481HP	SKU
https://www.thermofisher.com/order/catalog/product/SHKE481HP	LINK

- Centrifuge

Equipment

Sorvall™ Legend™ XT/XF Centrifuge Series	NAME
Thermo Scientific	BRAND
75004541	SKU
https://www.thermofisher.com/order/catalog/product/75004541	LINK

- Ultrasonic Processor 130W

Equipment

Ultrasonic Processor	NAME
130-Watt Ultrasonic Processor	TYPE
Cole-Parmer	BRAND
ML-04714-52	SKU
https://www.coleparmer.com/p/cole-parmer-130-watt-ultrasonic-processors/44347	LINK

- Ultrasonic Processor 750W

Equipment

750-Watt Ultrasonic Processor	NAME
CPX750	TYPE
Cole-Parmer	BRAND
ML-04711-60	SKU
https://www.coleparmer.com/p/cole-parmer-500-and-750-watt-ultrasonic-processors/16401	LINK

-Nanodrop

Equipment

NanoDrop™ One UV-Vis Spectrophotometer	NAME
spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-ONE-W	SKU
https://www.thermofisher.com/order/catalog/product/ND-ONE-W	LINK
Sample Volume (Metric): Minimum 1µL; Spectral Bandwidth: ≤1.8 nm (FWHM at Hg 254 nm); System Requirements: Windows™ 8.1 and 10, 64 bit; Voltage: 12 V (DC); Wavelength Range: 190–850 nm	SPECIFICATIONS

- FPLC system

Equipment	
ÄKTA pure	NAME
Protein purification system	TYPE
Cytiva	BRAND
29046665	SKU
https://www.cytivalifesciences.com/en/us/support/products/akta-pure-150-l-29046665	LINK

- Spectrophotometer UV/Vis
- Incubator (37°C)
- Water bath (60°C)
- Ultra Low–Temperature Freezer (-80°C)
- Freezer -20°C
- Refrigerator (4°C)
- Analytical balance

Other:
- Ice bath
- Microcentrifuge tubes
- Sterile 0.45 µm membrane filter
- 150 mL Superloop (Cytiva)
- Dialysis membrane
- Ultrafiltration tube (Amicon Ultra-15)

Equipment	
Amicon Ultra-15	NAME
PLTK Ultracel-PL membrane, 15 ML - 30 kDa cutoff	TYPE
Millipore	BRAND
UFC903024	SKU
https://www.merckmillipore.com/MX/es/product/Amicon-Ultra-15-Centrifugal-Filter-Unit,MM_NF-UFC903024?ReferrerURL=https%3A%2F%2Fwww.bing.com%2F&bd=1	LINK

- Image Lab 6.1 Software (Bio-Rad)

BEFORE START INSTRUCTIONS

Ensure to have all the necessary materials and reagents already cleaned, sterilized and filter (in case of the purification solutions).

Preparation of Bst expression cells

20h 45m

1 Transformation of chemically competent BL21 (DE3) cells with pKJE7 plasmid.

1.1 Add 1 µL of plasmidic DNA consisting of the pKJE7 plasmid from the Chaperone Plasmid Set **Takara Bio USA, Inc. Catalog #3340** to 50 µL of competent cells Chemically Competent E. coli One Shot™ BL21(DE3) **Invitrogen - Thermo Fisher Catalo #C600003** . Mix the cells gently and incubate On ice for 00:20:00 . 20m

1.2 Transfer the cells to a heat block at 42 °C and incubate for 00:00:53 . 53s

1.3 Transfer the cells immediately to an ice bath and incubate On ice for 00:05:00 . 5m

1.4 Add 250 µL of SOC medium at room temperature to the transformed cells and incubate at 225 rpm, 37°C, 01:00:00 . 1h

Note

SOC medium composition

	A	B
	Tryptone	2%
	Yeast extract	0.5%
	NaCl	10 mM
	KCl	2.5 mM
	MgCl2	10 mM
	MgSO4	10 mM
	Glucose	20 mM

Adjust to pH 7 and sterilize by filtration.

1.5 Plate 25 µL of transformed cell culture onto LB agar with the corresponding selective agent. Incubate the plates Overnight at 37 °C . 18h

Note

The pKJE7 plasmid requires [M] 30 µg/mL chloramphenicol as selective agent.

1.6 Select a single colony of transformed cells and inoculate in 3 mL Luria-Bertani (LB) medium supplemented with the selective antibiotic. Incubate 18h

Overnight at 180 rpm, 37°C .

1.7 Centrifugate the cell culture at 10000 x g, 4°C, 00:05:00 . Remove the supernatant and resuspend the cell pellet in 500 µL LB meduim . 5m

1.8 Add 500 µL of 30% v/v glycerol , mix by pipetting up and down and store at -80 °C . 5m

2 Preparation of chemically competent BL21 (DE3) cells harboring pKJE7 plasmid.

2.1 Take BL21(DE3) cells harboring pKJE7 plasmid from a frozen glycerol stock using a bacterial inoculating loop and inoculate 3 mL LB liquid medium with [M] 30 µg/mL of chloramphenicol . Incubate Overnight at 180 rpm, 37°C 18h

2.2 Inoculate 1 mL overnight culture in 100 mL LB medium with [M] 30 µg/mL chloramphenicol and incubate at 180 rpm, 37°C, 03:00:00 . 3h

> Note
>
> Monitor the cell growth by measuring the optical density **(OD) at 600 nm** and remove the cells from incubation when the OD reaches **0.3 to 0.4.**

2.3 Chill the cell culture On ice for 00:10:00 and centrifugate the cells at 4000 x g, 4°C, 00:10:00 . 20m

2.4 Gently resuspend the cell pellet in 8 mL of TFBI solution pre-chilled and incubate On ice for 00:45:00 . Centrifugate the cells at 4000 x g, 4°C, 00:05:00 . 50m

Note

TFBI Solution Composition

	A	B
	Potassium acetate	30 mM
	Rubidium chloride	100 mM
	Calcium chloride	10 mM
	Manganese chloride	50 mM
	Glycerol	15% v/v

Adjust to pH 5.8 with 1M acetic acid and sterilize by filtration.

2.5 Gently resuspend the cell pellet in 2.5 mL of TFBII solution pre-chilled and incubate On ice for 00:05:00. 5m

Note

TFBII Solution Composition

	A	B
	MOPS	10 mM
	Rubidium chloride	10 mM
	Calcium chloride	75 mM
	Glycerol	15% v/v

Adjust to pH 6.5 with 1M sodium hydroxide and sterilize by filtration.

2.6 Prepare aliquots of 50 µL of competent cells using microcentrifuge tubes previously 30m

chilled on an ice bath. Place the aliquots on dry ice until frozen and store at -80 °C.

3 Transformation of chemically competent BL21 (DE3)/pKJE7 cells with the pColdI-Bst plasmid, the expression vector for the large fragment of the *Bacillus stearothermophilus* DNA polymerase (Bst).

3.1 Add 1 µL of plasmidic DNA of [M] 100 ng/µL pColdI-Bst expression vector to 50 µL of competent cells BL21 (DE3)/pKJE7. For the transformation procedure go to step #1.

> **Note**
>
> The pColdI-Bst plasmid require [M] 100 µg/mL carbenicillin as selective agent and the pKJE7 plasmid require [M] 30 µg/mL chloramphenicol. Use LB medium supplemented with both antibiotics as selective media.

Small-scale screening cultures

4 Preparation of bacterial cultures for Bst expression.

4.1 Inoculate 5 µL glycerol stock of **BL21(DE3)/pKJE7/pColdI-Bst** or **BL21(DE3)/pKJE7** cells in 5 mL culture medium (LB or TB) supplemented with seletion agent(s). Incubate Overnight at 200 rpm, 37°C. 18h

4.2 Inoculate 500 µL overnight culture in 50 mL culture medium with antibiotic(s). Use LB or TB according to the medium used for the overnight culture. Incubate 200 rpm, 37°C for approximately 03:00:00. 3h

Note

Monitor the cell growth by measuring the optical density **(OD) at 600 nm** and remove the cells from incubation when the OD is between **0.6.**

4.3 Once the culture reaches an OD600 of 0.6, incubate the cell cultures On ice for 00:20:00 before adding the inducer (IPTG). 20m

5 Small-scale Bst expression under different induction conditions.

5.1 Induce the expression of the Bst under different conditions. Each treatment should be evaluated in triplicate. For example: 30m

	Strain	[IPTG]	Temperature	Medium
	BL21(DE3)/pKJE7*	0.5 mM	16°C	LB
	BL21(DE3)/pKJE7*	0.5 mM	37°C	LB
	BL21(DE3)/pKJE7*	0.5 mM	16°C	TB
	BL21(DE3)/pKJE7/pColdI-Bst	0 mM	16°C	LB
	BL21(DE3)/pKJE7/pColdI-Bst	0.1 mM	16°C	LB
	BL21(DE3)/pKJE7/pColdI-Bst	0.5 mM	16°C	LB
	BL21(DE3)/pKJE7/pColdI-Bst	1.0 mM	16°C	LB
	BL21(DE3)/pKJE7/pColdI-Bst	0 mM	37°C	LB
	BL21(DE3)/pKJE7/pColdI-Bst	0.5 mM	37°C	LB
	BL21(DE3)/pKJE7/pColdI-Bst	0.5 mM	16°C	TB

* BL21(DE3)pKJE7 strain is used as negative expression control.

5.2 Incubate at 16 °C or 37 °C according to each treatment at 180 rpm for 16:00:00. 16h

5.3 Centrifugate the cell cultures at 6000 x g, 4°C, 00:10:00. Discard the supernatant, remove all the liquid and leave the cell pellet as dry as posible. 10m

5.4 Weigh the centrifugation tube with the cell pellet (total weight). 10m

Note

Weigh the empty tube prior centrifugation and subtract it to the total weight to calculate the weight of the cell pellet and hence the biomass produced.

5.5 Resuspend the cell pellet in 5 mL lysis buffer A (LB-A) (pre-chilled). 5m

Note

Lysis buffer A (LB-A) composition

	A	B
	Tris-HCl pH 7.5	50 mM
	EDTA	0.5 mM
	2-mercaptoethanol	10 mM
	Tergitol NP-40	0.1% v/v
	Tween-20	0.1% v/v
	PMSF	3 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

5.6 Disrupt cells by ultrasonication at an amplitude of 40%. Apply five cycles of 00:00:15 on 3m 45s

and 00:00:30 off.

Note

Place the tubes On ice while processing.

Equipment

Ultrasonic Processor	NAME
130-Watt Ultrasonic Processor	TYPE
Cole-Parmer	BRAND
ML-04714-52	SKU
https://www.coleparmer.com/p/cole-parmer-130-watt-ultrasonic-processors/44347	LINK

5.7 Centrifugate at 6000 x g, 4°C, 00:15:00. Recover the supernatant (soluble protein fraction) and discard the pellet. 15m

5.8 35m

For protein clarification, incubate the supernatant at 60 °C for 00:20:00 in a water bath and centrifugate at 14500 x g, 4°C, 00:15:00. Recover the clarified supernatant and place it On ice.

6 Analysis of Bst expression.

6.1 Measure total protein concentration by measuring absorbance at 280 nm in a NanoDrop spectrophotometer. 3m

Equipment

NanoDrop™ One UV-Vis Spectrophotometer NAME

spectrophotometer TYPE

Thermo Scientific BRAND

ND-ONE-W SKU

https://www.thermofisher.com/order/catalog/product/ND-ONE-W LINK

Sample Volume (Metric): Minimum 1µL; Spectral Bandwidth: ≤1.8 nm (FWHM at Hg 254 nm); System Requirements: Windows™ 8.1 and 10, 64 bit; Voltage: 12 V (DC); Wavelength Range: 190–850 nm SPECIFICATIONS

6.2 Analyze all clarified supernatant samples by Tricine-SDS-PAGE electrophoresis through a 8% polyacrylamide gel. Load 100 µg protein sample per well. 4h

CITATION

Hermann Schägger (2006). Tricine-SDS-PAGE. Nature Protocols.

LINK

10.1038/nprot.2006.4

6.3 Select the best conditions for protein expression according to the results analysis (biomass, total protein production and electrophoretic profile).

Large-scale production of Bst

2h 6m 20s

7 Expression of recombinant Bst.

7.1 Inoculate 10 µL glycerol stock of **BL21(DE3)/pKJE7/pColdI-Bst** expression cells in 20 mL LB medium supplemented with [M] 100 µg/mL carbenicillin and [M] 30 µg/mL chloramphenicol. Incubate Overnight at 180 rpm, 37°C. 18h

7.2 Inoculate 10 mL overnight culture in 1 L LB medium with [M] 100 µg/mL carbenicillin and [M] 30 µg/mL chloramphenicol. Incubate at 200-220 rpm, 37°C, 03:00:00. 3h

> Note
>
> Monitor the cell growth by measuring the optical density **(OD) at 600 nm** and remove the cells from incubation when the OD reaches **0.6.**

7.3 Place the inoculum On ice for 00:30:00 and then add [M] 0.5 millimolar (mM) IPTG for induction. 30m

> Note
>
> Do not add any additional inducers. For expression of the chaperones contained in pKJE7 plasmid, the basal expression is enough to promote correct Bst enzyme folding.

7.4 Incubate at 180 rpm, 16°C, 16:00:00 for recombinant protein expression. 16h

8 Soluble protein fraction recovery and clarification.

8.1 Centrifugate at 6000 x g, 4°C, 00:12:00 to harvest cells. Discard the supernatant ensuring to remove all the liquid and leave the cell pellet as dry as posible. 12m

8.2 Weigh the centrifugation tube with the cell pellet (total weight). 3m

Note

Weigh the empty tube prior centrifugation and subtract it to the total weight to calculate the weight of the cell pellet and hence the biomass produced.

8.3 Store the cell pellet at -80 °C until use (just in case that the purification step is not performed immediately after expression).

8.4 Resuspend the cell pellet in 50 mL lysis buffer A (LB-A) (pre-cooled). If neccesary, defroze the cell pellet in an ice bath before adding the lysis buffer. 5m

Note

Lysis buffer A composition (LB-A)

	A	B
	Tris-HCl pH 7.5	50 mM
	EDTA	0.5 mM
	2-mercaptoethanol	10 mM
	Tergitol NP-40	0.1% v/v
	Tween-20	0.1% v/v
	PMSF	3 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

8.5 Disrupt cells by ultrasonication with an ultrasonic processor at an amplitude of 40% applying pulses of 00:00:10 of ultrasonication and 00:00:10 of pause during 00:04:00 . 4m 20s

Note

Place the sample On ice and keep it cold while processing.

Equipment

750-Watt Ultrasonic Processor	NAME
CPX750	TYPE
Cole-Parmer	BRAND
ML-04711-60	SKU
https://www.coleparmer.com/p/cole-parmer-500-and-750-watt-ultrasonic-processors/16401	LINK

8.6 Centrifugate at 11000 x g, 4°C, 00:30:00. Recover the supernatant (soluble protein fraction) and discard the pellet. 30m

8.7 Incubate the supernatant in a water bath at 60 °C for 00:20:00 for protein clarification. 20m

8.8 Centrifugate at 11000 x g, 4°C, 00:30:00. Recover the clarified supernatant and discard the pellet. 30m

Note

Place the supernatant On ice or store at 4°C until use.

Purification of recombinant Bst by FPLC

9 Sample preparation.

Note

Keep all protein samples On ice during the purification process to avoid degradation.

9.1 Filter the clarified supernatant through a 0.45 µm membrane.

9.2 Dilute the clarified supernatant with saline buffer at a radio of 1:1.

Note

Saline buffer composition (SB).

	A	B
	Tris-HCl pH 7.5	50 mM
	NaCl	100 mM
	PMSF	1 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

9.3 Load the diluted fraction onto a 150 mL Superloop (Cytiva). Store at 4 °C until use.

10 Immobilized metal affinity chromatography (Ni^{2+}-IMAC).

10.1 Connect a HisTrap HP 5mL **Cytiva Catalog #17524801** to a FPLC system.

Equipment

ÄKTA pure	NAME
Protein purification system	TYPE
Cytiva	BRAND
29046665	SKU
https://www.cytivalifesciences.com/en/us/support/products/akta-pure-150-l-29046665	LINK

10.2 Equilibrate the column with 8 column volumes (CV) of washing buffer-AI (WB-AI) at a flow of 2.5 mL/min.

Note

Washing buffer-AI composition (WB-AI).

	A	B
	Tris-HCl pH 7.5	50 mM
	NaCl	100 mM
	Imidazole	10 mM
	PMSF	1 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

10.3 Connect the Superloop charged with the protein fraction and load the sample onto the column at a flow of
2.5 mL/min.

10.4 Wash the column with 10 CV of WB-AI at a flow of 2.5 mL/min.

10.5 Wash the column with 10 CV of 2% elution buffer-AI (EB-AI) at a flow of 2.5 mL/min.

Note

Elution buffer-AI composition (EB-AI).

	A	B
	Tris-HCl pH 7.5	50 mM
	NaCl	100 mM
	Imidazole	500 mM
	PMSF	1 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

10.6 Elute the proteins by passing 5 CV of 100% EB-AI through the column using a flow of 2.5 mL/min.

10.7 Analyze all recolected fractions by a 8% Tricine-SDS-PAGE gel electrophoresis. Load 10 µL protein sample per well.

10.8 Pool all elution fractions carrying the recombinant Bst protein. Store at 4 °C until use.

11 Desalting step.

11.1 Connect a HiPrep 26/10 Desalting Column **Cytiva Catalog #17508701** to the FPLC system.

11.2 Wash the column with 2.5 CV of Mili-Q water. Then, equilibrate the column with 2 CV of desalting buffer-A (DB-A). For both steps use a flow of 10 mL/min.

Note

Desalting buffer-A composition (DB-A).

	A	B
	Tris-HCl pH 7.5	50 mM
	KCl	20 mM
	EDTA	1 mM
	DTT	1 mM
	PMSF	1 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

11.3 Load the sample onto the column at a flow of 5 mL/min.

11.4 Wash the column with 2 CV of DB-A for protein elution at a flow of 10 mL/min.

11.5 Analyze all collected fractions by qualitative Bradford assay using the Quick Start™ Bradford 1x Dye Reagent **BioRad Sciences Catalog #5000205**. Pool the fractions with higher protein concentration.

11.6 Load the pool of desalted fractions onto a 150 mL Superloop (Cytiva). Store at 4 °C until use.

12 Heparin affinity chromatography.

12.1 Connect a HiTrap Heparin HP affinity column **Cytiva Catalog #17040701** to the FPLC system.

12.2 Equilibrate the column with 10 CV of washing buffer-AII (WB-AII) at a flow of 2 mL/min.

Note

Washing buffer-AII composition (WB-AII).

	A	B
	Tris-HCl pH 7.5	50 mM
	EDTA	1 mM
	DTT	1 mM
	PMSF	1 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

12.3 Connect the Superloop charged with the protein fraction and load the sample onto the column at a flow of 2 mL/min.

12.4 Wash the column with 5 CV of WB-AII at a flow of 2 mL/min.

12.5 Elute proteins by washing the column with a linear gradient of 10 CV of elution buffer-AII (EB-AII). Use a flow of
2 mL/min.

Note

Elution buffer-AII composition (EB-AII).

	A	B
	Tris-HCl pH 7.5	50 mM
	KCl	1 M
	EDTA	1 mM
	DTT	1 mM
	PMSF	1 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

12.6 Analyze all collected fractions by Tricine-SDS-PAGE electrophoresis through a 8% polyacrylamide gel. Load 10 µL protein sample per well.

12.7 Pool all elution fractions carrying the recombinant Bst protein. Store at 4 °C until use.

13 Purified Bst enzyme concentration and formulation.

13.1 Load the purified Bst enzyme pool onto a dialysis membrane (pre-hydrated). Place the membrane into a beaker with precooled storage buffer-A (SB-A) at a ratio 1:50 (v/v).

Note

Storage buffer-A composition (SB-A).

	A	B
	Tris-HCl pH 7.5	10 mM
	KCl	50 mM
	EDTA	0.1 mM
	DTT	2 mM
	Glycerol	50% v/v

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

13.2 Dialyze Overnight at 4 °C with slow agitation.

13.3 Recover the dialized protein, load it onto an **Amicon Ultra-15ML - 30 kDa cutoff centrifugal filter.** Concentrate until a concentration equal or higher than [M] 1 mg/mL.

Equipment

Amicon Ultra-15

NAME

PLTK Ultracel-PL membrane, 15 ML - 30 kDa cutoff

TYPE

Millipore

BRAND

UFC903024

SKU

https://www.merckmillipore.com/MX/es/product/Amicon-Ultra-15-Centrifugal-Filter-Unit,MM_NF-UFC903024?ReferrerURL=https%3A%2F%2Fwww.bing.com%2F&bd=1

LINK

Note

Monitor protein concentration measuring absorbance at 280 nm using a NanoDrop spectrophotometer.

Equipment

NanoDrop™ One UV-Vis Spectrophotometer

NAME

spectrophotometer

TYPE

Thermo Scientific

BRAND

ND-ONE-W

SKU

https://www.thermofisher.com/order/catalog/product/ND-ONE-W

LINK

Sample Volume (Metric): Minimum 1µL; Spectral Bandwidth: ≤1.8 nm (FWHM at Hg 254 nm); System Requirements: Windows™ 8.1 and 10, 64 bit; Voltage: 12 V (DC); Wavelength Range: 190–850 nm

SPECIFICATIONS

13.4 Prepare aliquots of 50 µL of concentrated Bst enzyme .

13.5 Add [M] 0.1 % (v/v) triton X-100 to the enzyme aliquots and store at -20 °C .

13.6 Determine final protein concentration by measuring absorbance at 280 nm in a NanoDrop spectrophotometer.

13.7 Analyze the final Bst enzyme formulation by Tricine-SDS-PAGE electrophoresis through a 8 gel. Load 3 µL protein sample per well. Load 3 µL protein ladder Precision Plus Protein™ Unstained Protein Standards **Bio-rad Laboratories Catalog #1610363** .

13.8 Analyze the electrophoresis gel by densitometry using the **Image Lab 6.1 Software (Bio-Rad)**. Determine protein concentration for each Bst enzyme aliquot analyzed using the protein ladder as weight standard.

Note

The protein ladder Precision Plus Protein™ Unstained Protein Standards **Bio-rad Laboratories Catalog #1610363** includes three reference bands: the 50 KDa with 750 ng, the 20 KDa and 100 KDa bands with 150 ng each per 10 µL of the protein ladder mix.