Production of recombinant interferon inhibitor B18R

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Abstract

A protein B18R is encoded by Western Reserve (WR) strain of *Vaccinia virus*. B18R works as a decoy receptor for the type I interferons (IFNs), attaches to cell surfaces, binds IFNs without prominent species-specificity and prevents an establishment of the antiviral state. At a same time B18R has no effect on cell viability itself. Thereunder B18R has become an essential aid in cell technologies which use RNA-mediated gene delivery. Despite an established high demand for this protein in cell technologies and also attractive prospects for use in mammalian expression systems to maintain RNA-vectors, little information is available on obtaining of B18R. In majority of works utilizing it, this protein has not been produced in a pure form. Rather, conditioned media (CM) are collected from cell cultures infected with *Vaccinia virus* or transfected with a B18R mRNA. The CM containing secretory-expressed B18R are used without an isolation of B18R. As the production in cell cultures is expensive, a development of a bacterial expression system is warranted. Here is described the bacterial expression and a method for refolding of recombinant B18R. Multi-milligram quantities of recombinant B18R are easily produced and the product is biologically active despite bacterially-expressed B18R is not glycosylated. It has been successfully utilized to support persistence of a RNA vector derived from a genome of a RNA-virus in cell cultures capable of the antiviral state.

A synthetic gene B18R which encodes the aminoacid residues His20-Glu351 from the Genbank entry D01019 is used in this work. The B18R gene sequence is codon-optimized for the optimal expression in E.coli. A sequence of an expression plasmid pET28-B18R(HisTag) is deposited to the Genbank MG356786. The expression product has a hexahistidine tag at the N-terminus which is used for a metal-affinity purification at one step of the protocol.

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Guidelines

All chemical reagents mentioned in the protocol were purchased from Sigma-Aldrich and are of ACS or Reagent quality. A vector pET28c (Novagen) was used to construct an expression plasmid pET28-B18R(HisTag) which sequence is deposited to the Genbank entry MG356786. Cells of a *E.coli* strain BL21(DE3) were transformed with the plasmid pET28/B18R(HisTag) to produce the expression strain used in this protocol.

Before start

Required solutions:

Lysis buffer: 20% sucrose, 20 mM HEPES pH 7.5, 5 mM $MgCl_2$, 0.1% Triton-X100. Store at RT.

TE 1^X for preparing of solutions: 10 mM Tris-HCl pH 7.2, 1 mM EDTA. Store at RT.

Lysozyme stock: 100 mg/ml lysozyme (solution in 1^x TE). Store frozen at -20°C.

DNAse I stock: 1 mg/ml DNAse I (solution in 1^x TE). Store frozen at -20°C.

RNase stock: 10 mg/ml RNase A (solution in 1^x TE). Store frozen at -20°C.

PMSF stock: 0.2 M phenylmethylsulfonyl fluoride (PMSF) (solution in isopropanol). Store frozen at -20°C.

Wash buffer with sodium deoxycholate (SDC): 100 mM Tris pH 8.0, 2 mM EDTA, 0.5% SDC. Store at RT.

Buffer TE 1^X to wash inclusion bodies: 100 mM Tris pH 8.0, 2 mM EDTA. Store at RT.

Solubilization buffer: 50 mM Tris pH 10, 2% sodium lauroyl sarcosinate (SLS). Store at RT.

$CuSO_4$ stock: 0.2 M cupric sulfate ($CuSO_4$) solution in water. Store at RT.

Buffer A for metal-affinity chromatography (IMAC) containing 6M urea: 20 mM Na_2HPO_4 pH 7.4, 500 mM NaCl, 20 mM imidazole, 6M urea. Store at RT.

Buffer B for IMAC+6M urea: 20 mM Na_2HPO_4 pH 7.4, 500 mM NaCl, 500 mM imidazole, 6M urea. Store at RT.

Phosphate-buffered saline (PBS): prepared from tablets (Amresco Cat. E404). Store at RT.

Protocol

Step 1.

Prepare a night culture by inoculating of 5 ml of Luria-Bertani (LB) medium with kanamycin (50 ug/ml) with 5 ul of a cryo-culture of an expression strain. The expression strain is BL21(DE3) transformed with a plasmid pET28/B18R(HisTag). Incubate overnight at 37°C on a shaker (150 rpm).

Step 2.

Next day transfer the night culture to 1L of Luria-Bertani (LB) medium with kanamycin (50 ug/ml). Incubate at 37°C on a shaker (150 rpm) until an optical density at 600 nm is 0.8. At this point add isopropyl β-D-thiogalactoside (IPTG) to 1 mM. The induced culture should be grown for 6 hrs at 37°C (150 rpm).

Step 3.

Collect bacterial cells by centrifugation (8000 rpm, 10 min). Wash the bacterial biomass with water. For this purpose resuspend the pellet in 50 ml of water and pellet the bacterial cells by centrifugation (8000 rpm, 10 min).

Step 4.

Resuspend the bacterial biomass to obtain a 10% suspension in a lysis buffer (20% sucrose, 20 mM HEPES pH 7.5, 5 mM $MgCl_2$, 0.1% Triton-X100). Add 1/100 of the suspension volume of a lysozyme stock, 1/100 volume of DNAse I stock, 1/100 volume of RNase stock, 1/1000 volume of PMSF stock. Incubate the mixture at room temperature for 1 hr.

Step 5.

Add to the mixture a dry powder of sodium deoxycholate (SDC) to 0.5% (w/v). Dissolve SDC.

Step 6.

Treat the mixture on a ultrasonic disruptor. Make 20 pulses at 50% amplitude, each pulse 30 sec, with 3 min pauses, on ice bath. Make sure that a size of a sonotrode matches a volume of the mixture. The mixture should become not viscous.

Step 7.

Separate by centrifugation an insoluble fraction which contains inclusion bodies. For this purpose centrifuge the mixture (12000 rpm, 30 min, 4°C).

Step 8.

To remove bacterial lipids the pellet has to be washed once in a wash buffer with SDC (100 mM Tris pH 8.0, 2 mM EDTA, 0.5% SDC). For this purpose, resuspend the inclusion bodies to make a 10% suspension in the wash buffer with SDC. Sonicate the suspension briefly to disintegrate chunks of the pellet. Pellet the inclusion bodies by centrifugation as described.

Wash the inclusion bodies in a buffer TE (100 mM Tris pH 8.0, 2 mM EDTA). Resuspend the inclusion bodies (to 10%) in TE without SDC, sonicate briefly and re-pellet. Repeat the wash in TE one more time.

Step 9.

Weight the pellet of the washed inclusion bodies. Divide the pellet into portions by 70 mg. Store the inclusion bodies at -80°C before further processing.

(Note: typical yields of the inclusion bodies are 700 mg from 1 L of an induced culture)

Step 10.

Dissolve a portion (70 mg) of the inclusion bodies in 100 ml of a solubilization buffer (50 mM Tris pH 10, 2% sodium lauroyl sarcosinate (SLS)). Stir the suspension at room temperature for 2 hours

Step 11.

Add $CuSO_4$ to the protein solution to a final concentration 50 µM. Stir the protein solution in an open flask on a magnetic stirrer for 20 hrs at room temperature

Step 12.

Precipitate the protein from the solution. For this purpose, add 25 g of ammonium sulfate ($(NH_4)_2SO_4$) to 100 ml of the protein solution (final concentration of ammonium sulfate 40% w/v). Incubate the resulting solution for 2 hrs in ice bath. Precipitate the protein by centrifugation (10000 rpm, 20 min)

Step 13.

Dissolve the precipitate in 20 ml of a buffer A for metal-affinity chromatography (IMAC) containing 6M urea (20 mM Na_2HPO_4 pH 7.4, 500 mM NaCl, 20 mM imidazole, 6M urea). Remove undissolved material was by centrifugation.

Step 14.

Perform a metal-affinity chromatography. Use a column His GraviTrap (GE Healthcare) which can be prepared according to the manufacturer's instructions. Equilibrate the column by passing through it 10 ml of the buffer A+6M urea. Pass the protein solution (from the previous step) through the column. Wash the column with 20 ml of the buffer A+6M urea. Elute the protein by passing through the column of 10 ml of a buffer B for IMAC+6M urea (20 mM Na_2HPO_4 pH 7.4, 500 mM NaCl, 500 mM imidazole, 6M urea). Collect the eluate.

Step 15.

Subject the collected eluate to dialysis against 2 L of PBS. Make dialysis for two days at +8°C, with three buffer changes during the dialysis. Typically no precipitation is observedduring the dialysis.

Step 16.

Filter-sterilize the B18R solution through a 0.22-um filter and store at -80°C. The B18R solution must be clear. Measure the protein concentration using the Bradford method. Typical concentrations at this stage are >500 ug/ml.

Warnings

General rules for a laboratory safety should be followed.