Purification of His-tagged L11

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Wild type L11 has one cysteine at the 38th position. The protocol below describes a typical protein expression system for a genetic engineering protocol to allow for L11 fluorescent probe labeling. The desired double mutant L11 will introduce a cysteine at serine at the 87th position and the cysteine at 38th position will be changed to serine (**DM-L11:** S87C and C38S) such that it can be monitored and viewed with high resolution microscopy techniques.

Day1

His-tagged L11 is engineered into pET-14b vector, which is transformed into BL21 (DE3) pLysS cells.

- Prepare standard LB medium (4L) and LB/Amp plates (10). Autoclave growth media.
- Plate the transformed reaction onto the LB/amp plate and grow overnight at 37^0 C.
  (OR)
  Streak the glycerol stock on to a LB/Amp plate and grow overnight at 37^0 C.

Day2

Pick up the colony and grow a small scale culture [50ml of LB with Ampicillin (100µg/mL final)] overnight at 37^0 C.

Day3

Continuing to work under sterile conditions, inoculate and grow 4L of culture using the small culture approximately for 2 to 3 hours or until the $O.D_{600}$ reaches 0.5. Induce the culture with IPTG (1mM final) grow for another 3-4 hours.

Place the flasks on ice for few min. Harvest the cells by low speed centrifugation @ 5000RPM (Beckman Avanti JE Centrifuge-JLA 9.1 rotor) for 10 min. Discard the sup and store the pellet @ -80^{0}C until use.

Preparation of Buffers for purification under native conditions:

Lysis buffer(200ml):	**Stock**	**Qty.**
50 mM NaH_2PO_4 pH 8.0	0.5 M	20 ml
300 mM NaCl	3 M	20 ml
10 mM imidazole	1 M	2 ml
4 mM βME	14.3 M	56 µl
Adjust pH to 8.0 with NaOH		

Wash buffer(200ml):	**Stock**	**Qty.**
50 mM NaH_2PO_4 pH 8.0	0.5 M	20 ml
300 mM NaCl	3 M	20 ml
20 mM imidazole	1 M	4 ml
4 mM βME	14.3 M	56 µl
Adjust pH to 8.0 with NaOH		

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Elution buffer (200ml):	**Stock**	**Qty**
50 mM NaH_2PO_4 pH 8.0	0.5 M	20 ml
300 mM NaCl	3 M	20 ml
250 mM imidazole	1 M	50 ml
4 mM βME	14.3 M	56 µl
Adjust pH to 8.0 with NaOH		

Dialysis buffer – without $MgCl_2$ (1000ml):	**Stock**	**Qty**
20mM Hepes pH 7.0	1M	20 ml
0.5mM EDTA	0.5M	1 ml
6M Urea (M.Wt is 60.06)	Powder	360.36 g
6mM BME	14.3	0.419 ml
10mM NaCl	4M	2.5 ml

Dialysis buffer - with $MgCl_2$(1000ml):	**Stock**	**Qty**
20mM Hepes pH 7.0	1M	20 ml
0.5mM EDTA	0.5M	1 ml
6M Urea (M.Wt is 60.06)	Powder	360.36 g
6mM BME	14.3	0.419 ml
10mM NaCl	4M	2.5 ml
10mM $MgCl_2$	100mM	100 ml

Crude Purification on the Ni-NTA column:

- Load 12mL of Ni-NTA Superflow resin into a plastic flex column and let it settle.
- Flow DiH_20 through column 2 hours.
- Equilibrate the column with lysis buffer for 3 hour @ the flow rate of 0.8 ml/min.
- Lyse the cells with lysozyme (1mg/ml) in lysis buffer and resuspend the cells.
- Pass the lysate through the French press twice.
- Add 0.5 mL of DNaseI enzyme and incubate on ice for 1 hour.
- Centrifuge the lysate at 18000g speed for 30 min @ 4^0C and filter through a 0.45 µ filter and load the sup onto the pre-equilibrated column @ 0.3ml/min.
- Turn the UV (260 nm) on. Start the chart recorder 2cm/hour, 500mv.
- Wash the column with wash buffer O/N @ 0.2 ml/min.
- Elute the protein using elution buffer @ the flow rate 0.5 ml/min. and collect the fractions @ 2min/fraction=1ml.
- Pool the fractions and dialyze against the dialysis buffer without Magnesium.
- Repeat the dialysis using the dialysis buffer with Magnesium now.
- Takeout the protein and load onto the Source S FPLC column.

Additional Purification on Source S FPLC column:

Buffer A:		
20mM Hepes pH 7.0	1M	20 ml
0.5mM EDTA	0.5M	1 ml
6M Urea (M.Wt is 60.06)	Powder	360.36 g
6mM BME	14.3	0.419 ml
10mM NaCl	4M	2.5 ml
10mM $MgCl_2$	100mM	100 ml

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Buffer B:

20mM Hepes pH 7.0	1M	20 ml
0.5mM EDTA	0.5M	1 ml
6M Urea (M.Wt is 60.06)	Powder	360.36 g
6mM BME	14.3	0.419 ml
400mM NaCl	Salt	23.96 g
10mM $MgCl_2$	100mM	100 ml

Dialysis Buffer:

20mM Tris.Cl pH 7.8	1M	20 ml
0.5mM EDTA	0.5M	1 ml
4mM BME	14.3	0.279 ml
400mM KCl(F.Wt 74.55)	Salt	29.82 g
10mM $MgCl_2$	100mM	100 ml

Purification:

The protein purified from the Ni-NTA column is loaded onto the SourceS column and eluted using a gradient from buffer A to B (10-4100mM NaCl). The L11 is eluted out around 60mM NaCl. This protein is further concentrated on the centricon YM10 and dialyzed against dialysis buffer twice and stored at -80^0C.

Cleaning the NI-NTA column:

Wash the column with water.
Wash the column with 0.2M acetic acid. (Stock of glacial acetic acid conc. is17.4M)
(1.1mls. of glacial acetic acid into 100ml.water).
Wash with 30% glycerol.
Wash with water.
Wash with 30% ethanol.
Store the column in 4^0C.