C-LYTAG
Purification System

Ed. 1

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User's manual

Biomedal

C-LYTAG PURIFICATION SYSTEM

A system for immobilization and single step purification of recombinant proteins.

For research use only

Contents

1. About C-LYTAG system

Figure 1. C-LYTAG structure

C-LYTAG is a system comprising an integrated range of products for the expression and purification of C-LYTAG fusion proteins in *E. coli*. The method is based on the selective interaction of the choline binding domain of the *Streptococcus pneumoniae* N-acetylmuramoyl-L-alanine amidase LytA (C-LYTAG) with choline or choline analogues (tertiary or quaternary amines)(1,2). The macromolecular structure of C-LYTAG has been resolved (Figure 1) (3).

C-LYTAG system enables the single-step affinity purification of C-LYTAG fusion proteins using C-LYTRAP resin, a simple, selective and cost efficient chromatographic support. Binding conditions are gentle and do not involve covalent modifications, therefore the fusion protein is highly stable once bound to the resin. For this reason, the system can also be used for enzyme immobilization onto solid supports. C-LYTAG-fusion proteins are selectively eluted using choline-containing buffers.

C-LYTAG system yields high expression levels when induced, but maintains basal levels prior to induction. It integrates CASCADE™* technology, in which the expression of the fusion protein is controlled by linked regulatory circuits to amplify gene expression (Figure 2) (4). CASCADE™ employs two salicylate-responsive transcriptional activator proteins, NahR and XylS2. In the presence of salicylate, the NahR protein induces expression of XylS2 from the P_{sal} promoter. Salicylate also activates XylS2, which induces high-level expression of the gene of interest from the Pm promoter. The synergistic effect of using two transcriptional regulators in a sequential cascade amplifies expression levels nearly 20-fold compared to expression from either promoter individually.

NOTES

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The first portion of the cascade system (nahR/Psal::XylS22) is located in the chromosome of the *E. coli* REG-1 expression strain and the second (Pm/lacO/C-LytA) is contained in pALEX vectors.

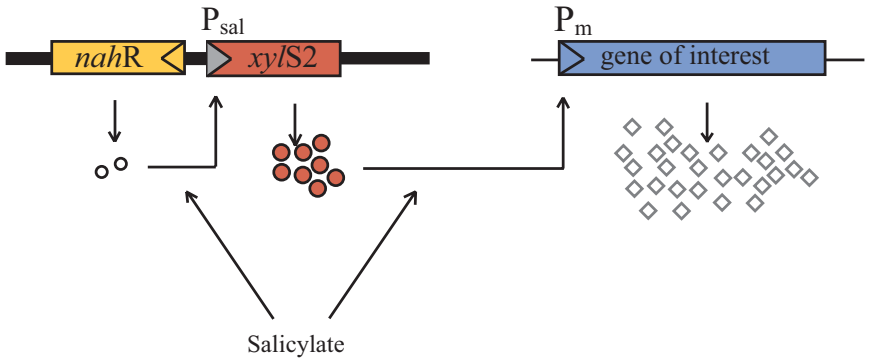


Figure 2. Cascade™ Expression system

The C-LYTAG Purification System (Cat. No. KT-3246) components are:

Component	Quantity	Storage	CAT. Nº.
pALEXa	8 µg (each)	-20°C	EV-3240
pALEXb			EV-3241
pALEXc			EV-3242
E. coli REG-1 strain	Stab	4°C	BS-3262
Inducer (salicylate, 1M)	25 ml	4°C	RS-3247
C-LYTRAP	30 ml	4°C; EtOH 20%	RS-3302
Choline chloride (3M)	20 ml	4°C	RS-3245

NOTE: C-LYTAG Purification System is shipped at room temperature. Upon arrival, store the components according to the directions in the table above.

* CASCADE™ is a trade mark of Active Motif, Inc., Carlsbad. The CASCADE™ expression system is patent pending and licensed by Active Motif, Inc. Commercial license available. Please contact us if you want more information about license agreement.

Main advantages of C-LYTAG Purification System

1. One-step purification from crude lysate to >95% pure protein.
2. Tightly regulated expression.
3. Resin is simple, inexpensive and reusable.
4. Compatible with virtually all common buffers.
5. Purified fusion protein can rebind to the matrix.
6. Elution buffers do not interfere with protein quantitation (Coomassie, UV-absorption, etc.).
7. The C-LYTAG moiety is easily refolded from inclusion bodies into a functional conformation.
8. No covalent modification of the protein is needed for efficient immobilization.
9. It has been successfully tested in many cases, and in a wide range of protein sizes: from small peptides (<10 aa) to large proteins (>1000 aa).

2. Strain and vectors

2.1. *E. coli* REG-1 strain

E. coli REG-1 strain contains the regulatory element *nah*R/P_{sal}::*xyl*S2 integrated into the chromosome.

Genotype

Mini-Tn*5*(*kan*^{r} *nah*R/P_{sal}::*xyl*S2) *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 (ara-leu)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G.

	Choline chloride 3M	Preparation of elution buffer.	20ml	RS-3245
	Salicylate (1M)	Inductor.	25ml	RS-3247
ANTIBIOTICS	Ampicillin	Cell culture tested.	5g	RS-3217
	Kanamycin	Cell culture tested.	5g	RS-3219
ANTIBODY	Anti C-LYTAG	Polyclonal antibody.	100 µl	AB-3238
OTHERS	Multibind 96 C-LYTAG	Multi-well format for multiple assays and high throughput screenings of biomolecules tagged with C-LYTAG	1 plate 5 plates	RS-3317 RS-3318
	C-LYTRAP Spin columns	Microcentrifuge columns containing C-LYTRAP resin	10 units	RS-3319
			15 units	RS-3320
			100 units	RS-3321
	Purification column	Empty columns for C-LYTRAP packing	1 unit (reusable)	RS-3322

4.5. References

1.- Sanz *et al* (1988). *FEBS Lett*. **232**, 308-312.
2.- Sánchez-Puelles *et al* (1992). *Eur. J. Biochem*. **203**, 153-159.
3.- Fernández -Tornero *et al* (2002). *J. Mol. Biol*. **321**, 163-173.
4.- Cebolla *et al* (2001). *Nucleic Acids Res*. **29**, 759-766.
5.- Cascade™. Instruction Manual. Active Motif.

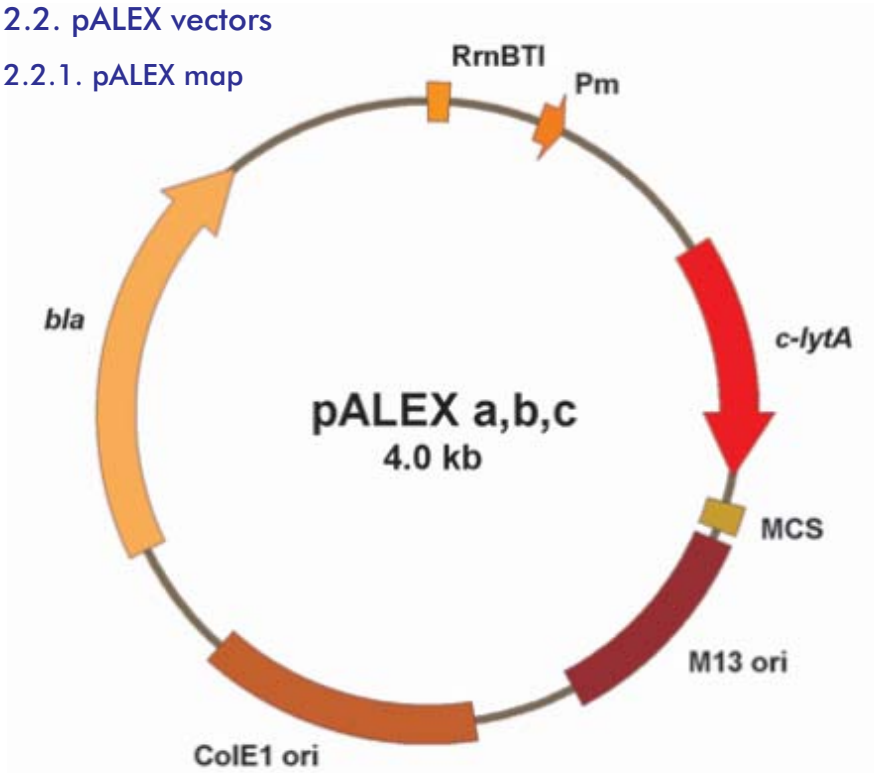
Fusion protein binds to C-LYTRAP but does not elute.	Elution is too fast.	Reduce the flow rate or let the elution buffer and resin in contact for 30 min.
	Non-specific interactions with the support.	Add 1.5% Triton X-100 to the elution buffer.
	Ionic interactions of the target protein with the support.	Increase the ionic strength in the choline-containing elution buffer (up to 1.5 M).
Fusion protein precipitation.		Add non-denaturing solubilizing agents (mild detergents, etc.).
	Choline absence.	Do not remove choline.
Fusion protein forms dimers.	Choline is present, which induces dimerization of C-LYTAG.	Remove choline.

4.4. Related products

PRODUCT		DESCRIPTION	FORMAT	Cat.No.
STRAINS	*E. coli* REG-1	Propagation of pALEX vectors and protein expression.	Stab	BS-3262
PRIMERS	C-LYTAG primer	Amplification by PCR (selection of positive clones) and sequencing of pALEX vectors.	2 nmol	PR-3281
VECTORS	pALEXa	Cloning and expression of C-LYTAG fusion proteins.	8 µg	EV-3240
	pALEXb	Cloning and expression of C-LYTAG fusion proteins.	8 µg	EV-3241
	pALEXc	Cloning and expression of C-LYTAG fusion proteins.	8 µg	EV-3242
	pALEX-*lacZ*	Positive control.	8 µg	EV-3243
	pALEX-gfp_{lav}	Positive control.	8 µg	EV-3239
	pALEX-*lip36*	Positive control.	8 µg	EV-3244
REAGENTS AND SOLUTION	TSS	Preparation of competent cells.	1.5 ml	RS-3215
			1.5 mlx5	RS-3216
	C-LYTRAP	Resin for C-LYTAG protein purification.	30 ml	RS-3302
			250 ml	RS-3316

2.2. pALEX vectors

2.2.1. pALEX map



2.2.2. General information

pALEX vectors are derivatives of pCAS vectors (5) and contain the 3´ moiety of the *Streptococcus pneumoniae lytA* gene (C-LYTAG protein) between P_m promoter/*lac* operator and the multiple cloning site.

pALEX vectors contain a multiple cloning site (MCS) that has seven unique restriction sites: *Bam*HI, *Xho*I, *Sac*I, *Bgl*II, *Kpn*I, *Bst*BI and *Hind*III (refer to vector map for details). They are available in all three reading frames (pALEXa, pALEXb and pALEXc), to facilitate cloning.

pALEX vectors also carry an enterokinase recognition sequence that enables removal of the C-LYTAG moiety from the fusion protein.

pALEX vectors carry as selection marker the *bla* gene, that confers ampicillin resistance.

2.2.3. Sequence and restriction analysis.

It is indicated:
ORANGE: Pm Promoter
PALE BLUE: Variable region in pALEXa, b and c vectors
RED: C-LYTAG sequence
BLUE: Enterokinase recognition sequence
■: pALEX Primer Forward (Cat. NO. PR-3433)
■: pALEX Primer Reverse (Cat. NO. PR-3434)

pALEXa vector

```
                                                        XbaI
201 TGCAAGAAGC GGATACAGGA GTGCAAAAAA TGGCTATCTC TAGAAAGGCC
251 TACCCCTTAG GCTTTATGCA ACAGAAACAA TAATAATGGA GTCATGACCA
301 TGACAATGCA CCTGGGGCTC GACTATATAG ATAGTCTCGT TGAAGAAGAT
351 GAGAACGAGG GCATCTACCG CTGCAAGCGC GAGATGTTCA CCGACCCTCG
401 GCTGTTCGAT TTAGAGATGA AACACATCTT TGAGGGCAAC TGGATTTATC
451 TCGCCCACGA GAGCCAGATT CCCGAGAAGA ACGACTATTA CACCACGCAG
501 ATGGGCCGGC AGCCGATATT CATCACACGC AACAAAGATG GTGAGCTGAA
                    EcoRI
551 TGCCTTCGTC AATGCCTGAA TTCGGAATTG TGAGCGGATA ACAATTCCTA
601 ACTTTATAGA TTACAAAACT TAGGAGGGTT TTTACCATGA TGGGCATTAG
                              RBS          M M    G I S
651 CCGTGAGCAG TTTAAGCATG ATATTGAGAA CGGCTTGACG ATTGAAACAG
      R E  Q   F  K  H  D   I  E  N   G  L  T   I  E  T  G
701 GCTGGCAGAA GAATGACACT GGCTACTGGT ACGTACATTC AGACGGCTCT
      W  Q  K   N  D  T  G  Y  W  Y   V  H  S   D  G  S
751 TATCCAAAAG ACAAGTTTGA GAAAATCAAT GGCACTTGGT ACTACTTTGA
    Y  P  K  D   K  F  E   K  I  N   G  T  W  Y   Y  F  D
```


4.2. Characteristics of C-LYTRAP resin

Binding capacity	0.5-3 mg/ml
Bead structure	6% highly cross-linked agarose
Bead size	45-165 µm
Recommended flow rate	1 ml/min
pH stability (<2 h)	1-14
pH stability (> 2 h)	3-12
Antimicrobial agent	Ethanol 20%
Storage	4°C for long time periods

4.3. Troubleshooting

PROBLEM	POSSIBLE CAUSE	RECOMMENDATION
No expression or low expression levels.	Gene is cloned into the wrong reading frame.	Review your cloning strategy to ensure that you choose the correct a, b or c vector.
	Culture temperature is too high or too low.	Change culture temperature.
	Concentration of inducer is too low.	Increase the concentration of inducer (0.5-5 mM salicylate).
	Messenger RNA instability or problems with translation (presence of multiple rare codons in the gene of interest).	Overexpression of the corresponding tRNA can help
	Insufficient cell breaking.	Check cell breaking conditions.
Improper protein folding.	Culture temperature is too high.	Use a lower temperature (30°C or less).
Fusion protein is not retained by the resin.	The amount of resin is not adequate.	Add more resin.
	Tertiary or quaternary amines in the extract buffer.	Dilute the extract so that amine concentration is below 10 mM.
	Steric hindrance between C-LytA and the fused protein.	Add a short peptide between both peptidic fragments.

4. Appendix

4.1. Composition of buffers

Cell resuspension buffer	20 mM sodium phosphate pH 7.0

BUFFERS FOR INCLUSION BODIES TREATMENT	
Buffer1:	guanidine chloride 6 M solution
Buffer 2:	20 mM sodium phosphate pH 7.0 150 mM choline chloride 1.5 M guanidine chloride
Buffer 3:	20 mM sodium phosphate pH 7.0 150 mM choline chloride 0.5 M guanidine hydrochloride
Buffer 4:	20 mM sodium phosphate pH 7.0

PURIFICATION BUFFERS	
Column equilibration buffer:	20 mM sodium phosphate pH 7.0
Washing buffer:	20 mM sodium phosphate pH 7.0 1.5 M NaCl
Re-equilibration buffer:	20 mM sodium phosphate pH 7.0 150 mM NaCl
Elution buffer:	20 mM sodium phosphate pH 7.0 150 mM choline chloride

NOTE :

a. Salt (up to 1.5M NaCl) may be added to the cell resuspension and column equilibration buffers. The presence of the salt excludes non C-LYTAG containing proteins and nucleic acids from binding to the resin. When determining optimal salt concentrations, the stability of the recombinant protein must be considered in relation to the ionic strength of the buffers.

b. Streptomycin sulfate (40 µg/ml) may be added to the resuspension buffer. Its addition causes the precipitation of nucleic acids, reduces the viscosity of the extract and decreases non-especific binding to the column.


```
801 CAGTTCAGGC TATATGCTTG CAGACCGCTG GAGGAAGCAC ACAGACGGCA
     S  S  G   Y  M  L  A  D  R  W   R  K  H  T  D  G  N
851 ACTGGTACTG GTTCGACAAC TCAGGCGAAA TGGCTACAGG CTGGAAGAAA
     W  Y  W   F  D  N  S  G  E  M   A  T  G  W  K  K
901 ATCGCTGATA AGTGGTACTA TTTCAACGAA GAAGGTGCCA TGAAGACAGG
     I  A  D  K  W  Y  Y  F  N  E   E  G  A  M  K  T  G

951 CTGGGTCAAG TACAAGGACA CTTGGTACTA CTTAGACGCT AAAGAAGGCG
     W  V  K  Y  K  D  T  W  Y  Y   L  D  A  K  E  G  A
    NcoI
1001 CCATGGTATC AAATGCCTTT ATCCAGTCAG CGGACGGAAC AGGCTGGTAC
      M  V  S  N  A  F  I  Q  S  A   D  G  T  G  W  Y
                                    EcoRI
1051 TACCTCAAAC CAGACGGAAC ACTGGCAGAC AGGCCAGAAT TCACAGTAGA
     Y  L  K  P  D  G  T  L  A  D   R  P  E  F  T  V  E
                                  NheI
1101 GCCAGATGGC TTGATTACAG TAAAAGCTAG CATGACTGGT GGACAGCAAA
      P  D  G  L  I  T  V  K  A  S   M  T  G  G  Q  Q  M
                                EK Cleavage Site▽ ClaI  BamHI SacI
1151 TGGGTCGGGA TCTGTACGAC GATGACGATA AGGATCGATG GGGATCCGAG
      G  R  D  L  Y  D  D  D  D  K   D  R  W  G  S  E
     BglII  PvuII                  BstBI    NotI
    XhoI   PstI     KpnI  NdeI   EcoRI   HindIII
1201 CTCGAGATCT GCAGCTGGTA CCATATGGGA ATTCGAAGCT TGCGGCCGCC
     L  E  I  C  S  W  Y  H  M  G   I  R  S  L  R  P  P

1251 CAGCTTGCTG GCGTACCGTT CCTGTCTAAA ATCCCTTTAA TCGGCCTCCT
      S  L  L  A  Y  R  S  C  L  K   S  L  *
```

Version B variable region: GATCCGAG
Version C variable region: CATCGATG GATCCGAC

Note: Restriction sites *ClaI*, *PstI*, *EcoRI* and *NotI* are not uniques in the plasmid. You should take into account this in order to design your cloning strategy

RESTRICTION ENZYMES THAT DO NOT CUT pALEX vectors

AarI	*AscI*	*BmgBI*	*BspEI*	*EcoNI*	*MluI*	*PmeI*	*SalI*	*SpeI*
AccI	*AsiSI*	*BplI*	*BspMI*	*EcoRV*	*MscI*	*PmlI*	*SapI*	*SphI*
AfeI	*AvrII*	*Bpu10I*	*BsrGI*	*FalI*	*NruI*	*PshAI*	*SbfI*	*SrfI*
AflII	*BbvCI*	*BseRI*	*BssHI*	*FseI*	*NsiI*	*Psp0MI*	*SexAI*	*SwaI*
AgeI	*BclI*	*BsgI*	*BstEII*	*FspAI*	*PacI*	*RsrII*	*SfiI*	*Tth111I*
AleI	*BfrBI*	*BsiWI*	*BstZ17I*	*HpaI*	*PflMI*	*SacI**	*SgrAI*	*XcmI*
ApaI	*BlpI*	*BsmBI*	*EcoICRI**	*MfeI*	*PfoI*	*SacII*	*SmaI*	*XmaI*

* pALEXc only

RESTRICTION ENZYMES THAT CUT ONCE pALEX vectors

AatII	*BamHI*	*BsmFI*	*EcoICRI*	*NcoI*	*PsrI*	*SnaBI*
Acc65I	*BbeI*	*BstAPI*	*FspI*	*NdeI*	*PvuI*	*StuI*
AflIII	*BglI*	*BstBI*	*HincII*	*NheI*	*PvuII*	*StyI*
AhdI	*BglII*	*Bsu36I*	*HinDIII*	*NspI*	*SacI*	*XbaI*
AloI	*BmtI*	*BtgI*	*KasI*	*PciI*	*SanDI*	*XhoI*
AlwNI	*BsaXI*	*DroIII*	*KpnI*	*PpuMI*	*ScaI*	*ZraI*
BaeI	*BsmI*	*EorI*	*NarI*	*PsiI*	*SfoI*	

3. Protocols

3.1. Cloning into pALEX vectors and host strain transformation (*E. coli* REG-1)

The cloning strategy must take into consideration that:

- The DNA encoding the target protein must be cloned in frame with the start codon (ATG) of the C-LYTAG coding sequence (see C-LYTAG and MCS sequence). Determine which restriction sites will be used for cloning and then choose the pALEX vector that will preserve the reading frame at the 5´ end.
- A stop codon must be included to terminate protein translation. pALEX vectors **do not** include a stop codon.

If contaminants are present:

1. Wash with 5 volumes NaOH 1 M.
2. Wash with 5 volumes of water.
3. Wash with 5 volumes 70% ethanol.
4. Equilibrate with 2 or 3 column volumes of column equilibration buffer, or 20% ethanol and store at 4°C, if the column is not used for a long period of time (more than a week). The colum may be reused 5-10 times.

GENERAL NOTES:

a. The purification can be performed at 4°C or room temperature, depending on the stability of the target protein.

b. The re-equilibration procedure reduces the presence of salts in the purified fraction and may eliminate the presence of nucleic acids or other components of the crude extracts which could otherwise be eluted by the small, but significant, additional increase in the ionic strength produced when choline is added.

c. The presence of choline analogues (tertiary and quaternary amines) in a relatively high concentration (>20 mM) may prematurely elute the target protein.

d. The target protein may be eluted using lower choline concentrations (from 30 mM choline), but the collected sample is more diluted.

e. Protein quantitation: C-LYTAG has an $E^{0.1\%}_{(280nm)}=3.72$ and a MW=21287 Da.

3.3.5. Choline elimination

Choline is basically a non-reactive and optically transparent molecule, therefore removal from the purified sample may not be necessary, thus avoiding additional purification steps (desalting, dialysis, chromatography, etc.). Besides, C-LYTAG is more stable when choline is present, although choline may induce dimerization of the fusion protein via C-LYTAG (3) and it may be desirable to remove the eluent in some cases.

To eliminate the choline, dialyze against phosphate buffer 20 mM pH 7.0 plus 50 mM NaCl. The dialysis can be done at temperature range between 4°C-room temperature. When the sample is very concentrated choline elimination may cause protein precipitation.

Once choline is removed, or its concentration is diluted below 10mM, the C-LYTAG fusion protein is able to bind again to C-LYTRAP.

3.3.6. Enterokinase cleavage

C-LYTAG fusion proteins carry an enterokinase cleavage site that allows for removal of the C-LYTAG moiety. Enterokinase is a specific protease that cleaves at Asp-Asp-Asp-Asp-Lys- after the lysine residue. The amount of enzyme required to cleave a fusion protein in a 16 h reaction at room temperature ranges from 0.001% to 0.5% (w/w). Depending on the particular fusion protein, the amount of protease can be adjusted within this range. Follow your enterokinase manufacturer´s instructions for an optimal cleavage of the fusion proteins.

3.3.7. Column regeneration and storage

If contaminants are not detected:

1. Wash adding 2-3 volumes of elution buffer.
2. Equilibrate with 5 column volumes of column equilibration buffer, or 20% ethanol if the column is not used for a long period of time (more than a week). Store at 4 °C.

- Transformation of *E. coli* REG-1 strain may be performed using standard methods. Biomedal recommends TSS solution (Cat.No. RS-3215/16), a fast and simple system to prepare competent cells (for information www.biomedal.es).

3.2. Protein expression

Optimal expression conditions should be determined for each particular recombinant protein with small scale cultures before attempting large scale expression procedures. Expression can be optimized by varying inducer concentration (0.5-5 mM salicylate), temperature (22-37°C) and time of induction (4 h-overnight).

1. Inoculate a pre-culture with a single, freshly transformed colony. Add the appropriate antibiotics (25 µg/ml kanamycin and 100 µg/ml ampicillin) to the pre-culture and incubate overnight at 37°C with shaking (225 r.p.m.).
2. Inoculate a culture with a 1:100 dilution of the pre-culture. Add the appropriate antibiotics and grow the culture at 37°C, 225 r.p.m. for 2-2.5 hours (until the OD_{600} is 0.2-0.3).
3. Add the expression inducer (salicylate) to a final concentration of 2 mM.
4. Grow induced culture for 5 h at 30°C and 225 r.p.m.
5. Harvest the culture by centrifugation at 4000 x *g* for 15 min at 4°C. Remove the supernatant and store the pellet at –20°C until needed.

3.3. Protein purification

Optimal purification conditions should be determined for each particular recombinant protein with small scale cultures before attempting large scale purification procedures.

The following protocol is recommended for cultures of 0.5-4 L. Volumes of resin and buffers can be scaled in this range of culture volume. Conditions for larger and smaller culture volumes must be optimized.

3.3.1. Column preparation

C-LYTRAP is supplied pre-swollen as a 75% slurry, stored in 20% ethanol. Pack the required amount of resin (8 ml C-LYTRAP per litre of culture) in an appropriate column and equilibrate with 2-3 volumes of column equilibration buffer. Ensure that the equilibration buffer completely replaces the 20% ethanol buffer.

NOTE ▶ a. During shipping and storage, the resin will settle. We recommend thoroughly resuspending it before pipetting.

b. The column must not be allowed to run dry. If it run dry, resuspend the resin in column equilibration buffer and repeat the packing.

3.3.2. Extract cell preparation

1. Resuspend the pellet (obtained in step 5 of Section 3.2) in 50 ml of cell resuspension buffer per litre of culture. Keep the sample on ice and disrupt the cells by sonication or using a French press.
2. Centrifuge at 9000 x *g* for 20 minutes to pellet the cell debris.
3. Remove the supernatant (crude extract) to a fresh container and save on ice.
4. Some proteins form inclusion bodies when they are expressed at high levels in bacteria. At this point, it is important to determine whether the expressed protein is soluble or is located in the insoluble fraction (inclusion bodies) by means of SDS-PAGE or Western–blot. If the expressed protein is located in the insoluble fraction, the pellet must be solubilized following the procedure below: (see Section 4 for composition of buffer)
    1. Resuspend the pellet (obtained in step 2) using 10 ml of Buffer 1.
    2. Centrifuge at 9000 x *g* (10 min) and discard pellet.
    3. Dialyze for 4 h against 500 ml of Buffer 2 at 20°C.
    4. Dialyze for 3 h against 500 ml of Buffer 3 at 20°C.
    5. Dilute 1:10 with 90 ml of Buffer 4.
    6. Centrifuge at 9000 x *g* (10 min). The supernatant is ready to be loaded onto the C-LYTRAP column.

NOTE ▶ Although C-LYTAG domain does not form inclusion bodies by itself, the hybrid protein may do so. This protocol has been succesfully tested in many cases, but it may need readjusting depending on each particular protein (addition of detergents, variation of temperature, etc.). On the other hand, direct dialysis of the resuspended precipitate from point 1 above against Buffer 4 may also yield good results.

5. Load the crude extract or the solubilized inclusion bodies onto the C-LYTRAP column. We recommend a flow rate of not higher than 1 ml/min in order to allow a thorough contact between extract and resin.

3.3.3. Column washing and elution

1. Wash the column with 10 volumes of washing buffer or until OD_{280} is less than 0.01.
2. Re-equilibrate with 2 volumes of re-equilibrating buffer.
3. Elute the fusion protein with 3-4 volumes of elution buffer. Collect fractions and analyze them to detect the presence of the fusion protein by means of colorimetric assay, measurement of OD_{280} or using anti C-LYTAG antibodies Biomedal (Cat. No. AB-3238).

3.3.4. Batch purification

Proteins may be purified on C-LYTRAP resin in either a batch or a column procedure. When the fusion protein is expressed at low levels or the crude extract is viscous, protein yields can be improved using batch purification. This procedure entails binding the protein to the C-LYTRAP resin in solution, mixing the resin and the crude extract in a flask and shaking gently for at least 1 h. The washing and elution may be carried out either in batch or in a column procedure, as is described in Section 3.3.