THE SUPERIOR SYSTEM FOR HIGH-LEVEL PROTEIN EXPRESSION

Affinity™

Protein Expression and Purification System

High-Level Expression and Easy Purification

Finally, high levels of protein expression and purification can be achieved without the harsh elution conditions and large purification tags that plague other systems! The Affinity™ protein expression and purification system provides the highest levels of protein expression of any prokaryotic system available. The T7 RNA polymerase-based pCAL vectors express cloned proteins as fusions with the calmodulin-binding peptide (CBP) tag. The CBP-tagged proteins are easily purified after only one pass through calmodulin resin. Both binding and elution of the CBP-tagged proteins are at neutral pH using mild buffer conditions. This means that the Affinity system provides an easy, gentle and effective method for protein expression and purification.

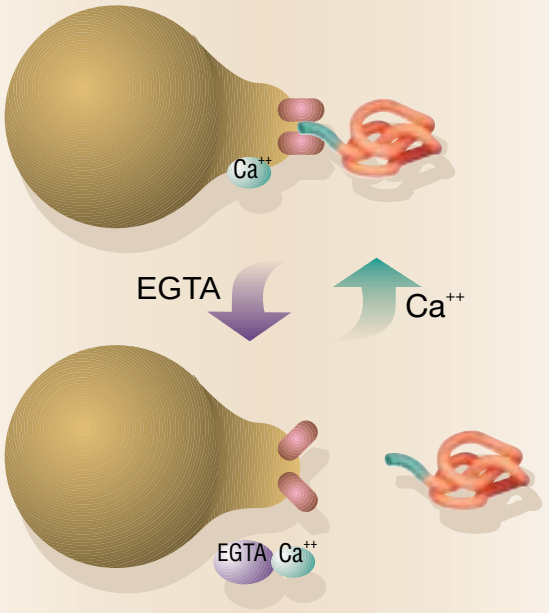
Easy Purification

Small Tag with Gentle Elution Conditions

The Affinity™ system's pCAL vectors feature the novel calmodulin-binding peptide (CBP) affinity tag. CBP-tagged proteins can be purified to near homogeneity with a single pass through calmodulin affinity resin. The CBP tag eliminates many of the problems common with 6xHis and glutathione-*S*-transferase (GST) affinity tags in *E. coli*. The CBP tag binds to calmodulin resin in the presence of low concentrations of calcium and is eluted in the presence of 2 mM EGTA at neutral pH. This makes it an excellent alternative to the 6xHis affinity tag, that requires harsh elution conditions such as low pH or high concentrations of imidazole. Because the CBP tag is only 4-kDa, it has less effect on the physical characteristics of the protein of interest than the larger 26-kDa GST affinity tag. This means that proteolytic removal of the CBP tag is not needed as often. Expressed proteins also include a thrombin protease cleavage site for optional CBP tag removal.

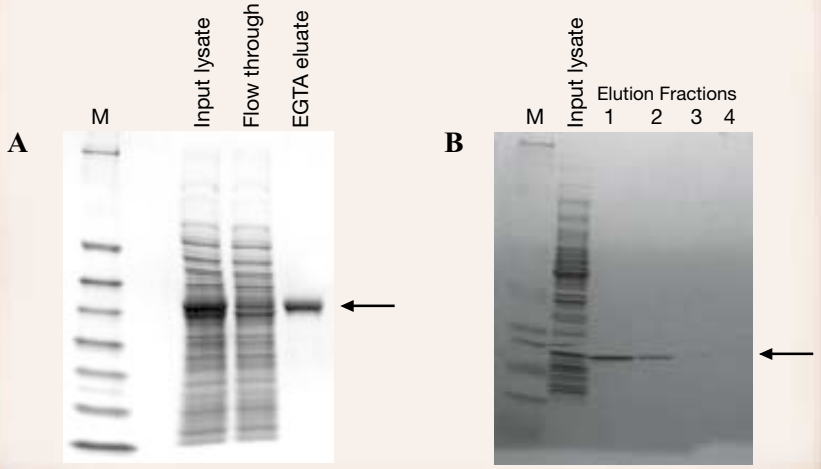
	CBP Affinity Tag	6xHis	GST
Small Tag	★	■	
Mild Elution	★		■
One-Column Purification	★	■	■

Binding of CBP Tag to Calmodulin Resin



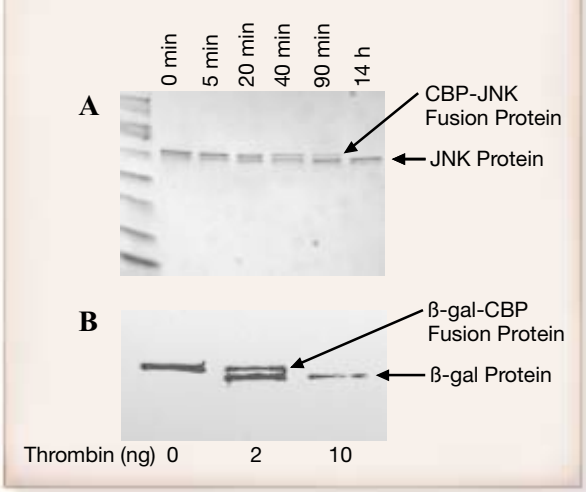
In the presence of calcium at neutral pH, the CBP tag binds to the calmodulin resin. The CBP tag is eluted with 2 mM EGTA at neutral pH.

Affinity Purification of Fusion Proteins Tagged at C and N Termini



Two different proteins were cloned into the pCAL-n and pCAL-kc vectors and transformed into BL21(DE3)pLysS competent cells. Cultures were induced with IPTG and lysed.
Total lysate, flow-through and eluted fractions were run on a SDS-PAGE gel and stained with Coomassie® Brilliant Blue Stain.
Panel A: The JNK protein was cloned into the pCAL-n vector.
Panel B: The GRB2(SH2) was cloned into the pCAL-kc vector.

Proteolytic Removal of CBP Affinity Tag



A. Affinity-purified JNK-CBP fusion protein (10 mg) was incubated with 20 ng of thrombin in thrombin-cleavage buffer. Samples were analyzed by 10% SDS-PAGE gel.

B. Affinity-purified b-gal-CBP fusion protein (490 ng) was incubated with various amounts of thrombin for 2 hours. Samples were analyzed by 10% SDS-PAGE gel.

Highest Levels of Protein Expression

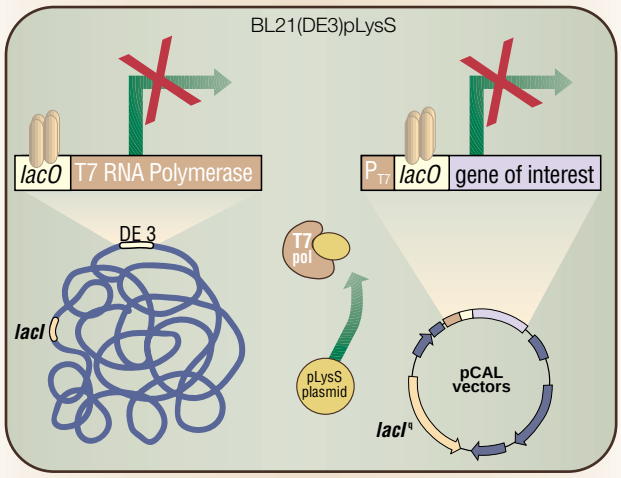
With Tight Regulation

The expression vectors in the Affinity™ system have been designed to provide the highest levels of protein expression, coupled with tight regulation. pCAL vectors are derived from the T7 RNA polymerase-based pET-11 series vectors.* These vectors contain the T7/*lacO* promoter and a plasmid-borne copy of the *lacI*^{q} gene to allow tight repression in *E. coli*. In the presence of IPTG, T7 RNA polymerase is expressed in the *E. coli* BL21(DE3) or BL21(DE3)pLysS host cells. The T7 RNA polymerase binds to, and transcribes from, the T7 promoter on the pCAL vector. High-level translation is from the strong T7 gene 10 ribosome-binding site. Transcription and translation are highly effective and after only a few hours, the target protein may constitute a majority of the cellular protein.

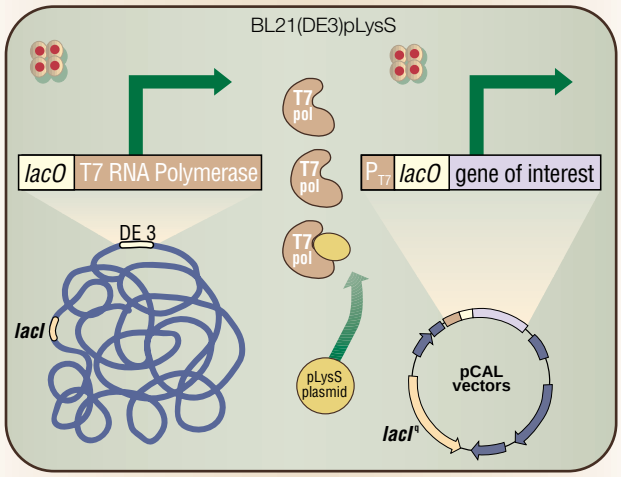
Versatile Expression

Using the pCAL vectors, recombinant proteins can be expressed as C- or N-terminal fusion proteins to the CBP affinity tag. The CBP portion of the expressed proteins may be labeled *in vitro* with cyclic AMP-dependent protein kinase and g-^{32}P. The presence of the kemptide sequence expressed from the pCAL-kc vector allows *in vitro* protein labeling in experiments where the CBP tag will be removed. With the Affinity system, you can choose the pCAL vector that best suits your research needs. All three of the pCAL vectors are also available separately.

Control of Expression

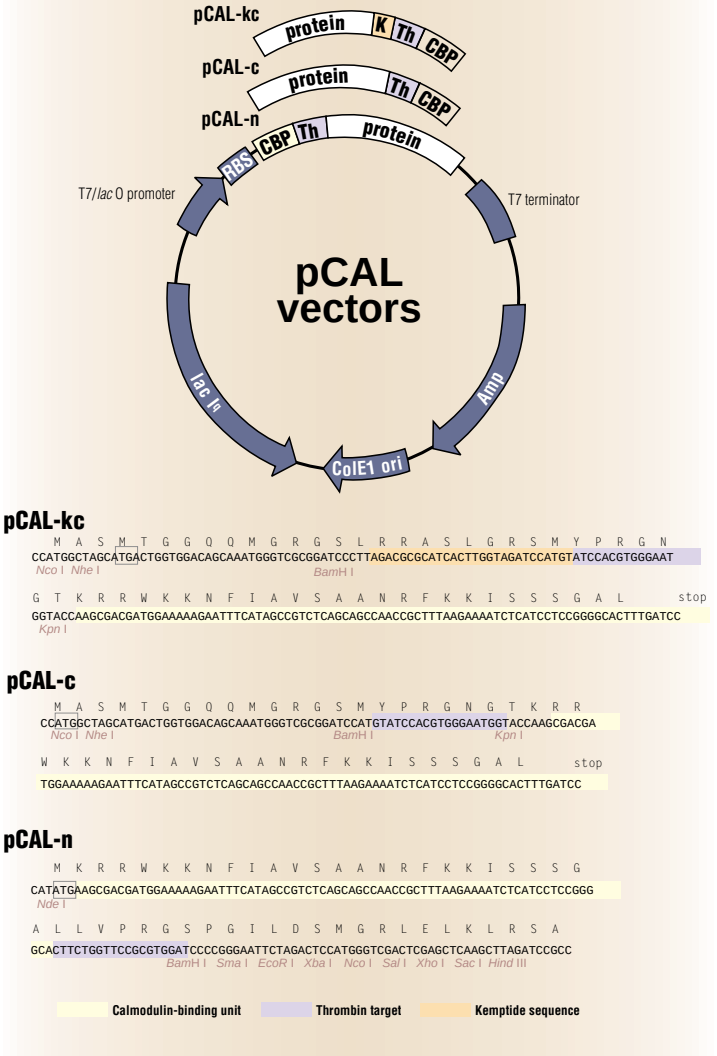


Repression: Transcription of both T7 RNA polymerase and gene of interest is blocked by Lac repressor protein ( ). pLysS plasmid produces T7 lysozyme ( ), an inhibitor of T7 polymerase.



Induction: IPTG binds Lac repressor protein ( ) derepressing the *lac* operator. High expression of T7 polymerase overrides repression of T7 lysozyme, resulting in high-level expression of the gene of interest.

pCAL Vectors



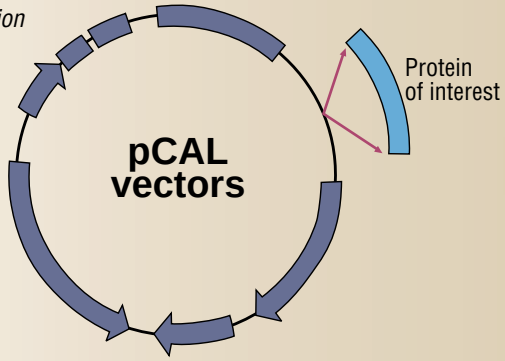
The p-CAL-n vector was derived from the pET-11a vectors and contains the CBP affinity tag plus the thrombin-cleavage site positioned for N-terminal fusion to the recombinant protein of interest.

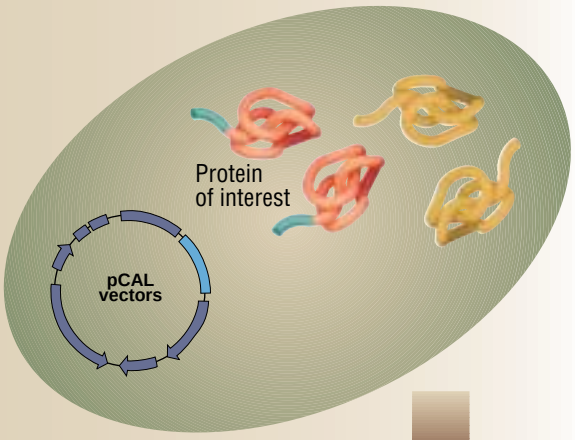
The pCAL-c and pCAL-kc vectors were derived from the pET-11d parent vector and contain the CBP affinity tag and thrombin-cleavage site positioned for C-terminal fusion. The pCAL-kc vector also contains a kemptide sequence positioned for C-terminal fusion.

Easy Protein Expression and Purification

Expression of your protein

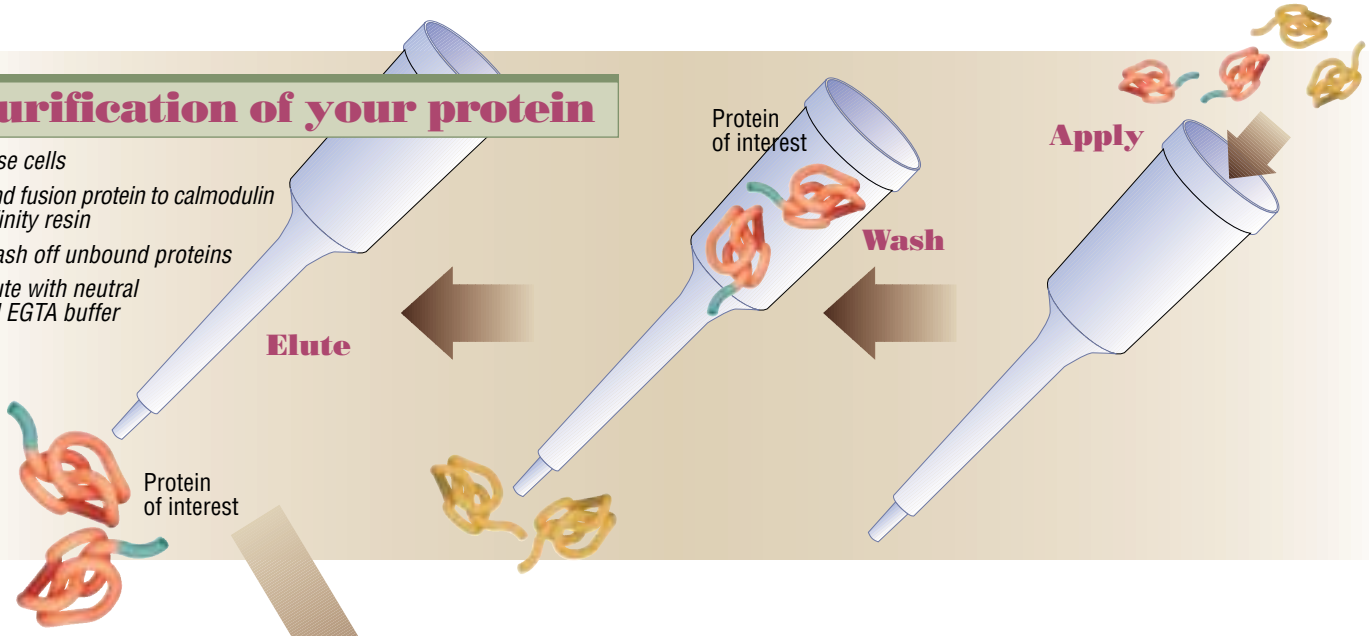
- *Clone your gene of interest into one of the pCAL vectors and transform into one of the BL21 competent cells*
- *Induce expression of your gene of interest*





Purification of your protein

- *Lyse cells*
- *Bind fusion protein to calmodulin affinity resin*
- *Wash off unbound proteins*
- *Elute with neutral pH EGTA buffer*



Analysis of your protein

- *Observe protein expression with Affinity CBP fusion detection kit*
- *Cleave CBP tag from your protein with thrombin*

STRATAGENE

Competent Cells for Protein Expression

Epicurian Coli® BL21 Competent Cells

The Epicurian Coli® BL21 competent cells have been specifically designed for use in high-level protein expression. These cells lack the Lon and OmpT proteases for optimal expression of your recombinant protein.

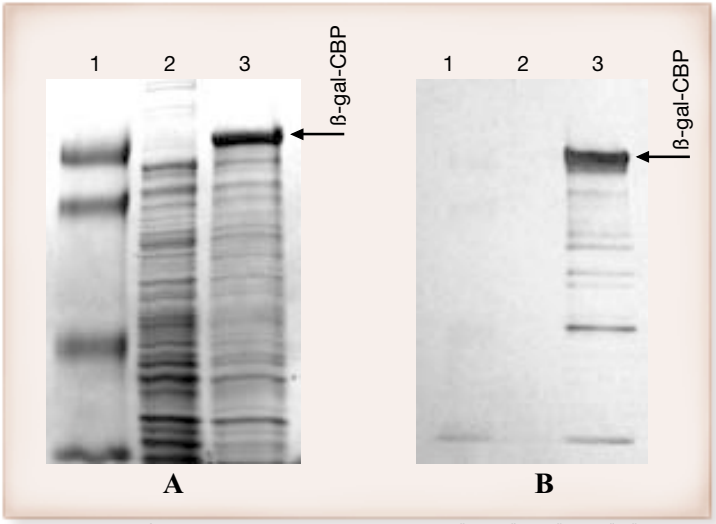
Three different strains that vary in repression levels can be used to induce recombinant protein. See the table below to select the strain and induction method for optimal expression of your protein.

Epicurian Coli® BL21 Competent Cells	Method of Induction	Advantages
BL21(DE3)	IPTG induction of T7 RNA polymerase from *lacUV5* promoter	• Use with nontoxic proteins • All purpose strain for high-level expression and ease of induction
BL21(DE3)pLysS	IPTG induction of T7 RNA polymerase from *lacUV5* promoter	• Use with toxic and nontoxic proteins • Tight control; pLysS plasmid codes for T7 lysozyme, a natural inhibitor of T7 RNA polymerase, to prevent leaky expression
BL21	Infection with lambda bacteriophage CE6 expressing T7 RNA polymerase; Can be used as a general protein expression host with vectors other than pET or pCAL vectors	• Use only with extremely toxic proteins • No expression until infection with lambda CE6

Rapid, Sensitive Detection

Affinity™ CBP Fusion Protein Detection Kit

Stratagene's Affinity™ CBP fusion protein detection kit allows rapid and sensitive detection of fusion proteins without the need for specific antibodies. It can quickly detect as little as 10 ng of CBP-tagged recombinant protein. Based on a simple immunoblotting format, the kit uses a highly specific biotinylated calmodulin probe and streptavidin alkaline phosphatase as the detection reagent. Tagged proteins can be easily detected on a western blot. Use the Affinity detection kit to monitor the expression levels, assess the stability of CBP fusion proteins and to study calmodulin-mediated signal transduction pathways.



Detection of CBP Fusion Using Biotinylated Calmodulin

b-galactosidase was cloned into the pCAL-c vector and transformed into Epicurian Coli® BL21(DE3)pLysS competent cells. Uninduced and induced cultures were lysed and electrophoresed on an SDS-PAGE gel in duplicate. Panel A was stained with Coomassie Brilliant Blue Stain, while panel B was transferred to nitrocellulose and probed using the Affinity CBP fusion detection kit.
Lane 1: Markers
Lane 2: Uninduced
Lane 3: Induced

Complete System

The Affinity™ protein expression and purification system contains most of the reagents necessary for expression and purification of recombinant protein: your choice of pCAL vectors, EGTA for elution of recombinant protein, BL21(DE3)pLysS competent cells for expression of recombinant protein and thrombin for optimal cleavage of the CBP tag from recombinant protein. The pCAL vectors, calmodulin resin and BL21(DE3)pLysS competent cells are also available separately.

Affinity™ Protein Expression and Purification System	
Contents Your choice of pCAL vector Epicurian Coli® BL21(DE3)pLysS competent cells Thrombin Calmodulin affinity resin EGTA	
Affinity system with pCAL-kc vector	204300
Affinity system with pCAL-c vector	204301
Affinity system with pCAL-n vector	204302

Affinity™ Expression Vectors	
Contents Your choice of pCAL vector *E. coli* XL1-Blue strain	
pCAL-kc vector	214300
pCAL-c vector	214301
pCAL-n vector	214302

Calmodulin Affinity Resin	
Contents 10 ml of resin	214303

Epicurian Coli® BL21 Competent Cells	
BL21(DE3) (5 x 0.2 ml)	200131
BL21(DE3)pLysS (5 x 0.2 ml)	200132
BL21 (5 x 0.2 ml)	200133

Affinity™ CBP Fusion Detection Kit	200370
Contents Biotinylated calmodulin Nitroblue tetrazolium (NBT) BCIP Tween® 20 Streptavidin alkaline phosphatase	

References

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