pALEX2-Ca

(Cat. No. EV-3435)

For research use only.

1. About BIOMEDAL C-LYTAG System.

pALEX2-Ca is a new generation of CASCADE™ * expression vector for the production of C-terminal fusion to C-LYTAG sequence. The final expression vector with the inserts has to be introduced in CASCADE™ expression hosts such as *E. coli* REG1 or MEDAL-GOLD (Cat. No. BS-3262 and BS-3432), that contain the *nahR-PsaI::xylS2* regulatory module integrated into the chromosome. The main features of this expression plasmid are:

- Improved version of *Pm* promoter with lower basal transcriptional activity.
- Short artificial 5' untranslated sequence with optimized efficiency for translation.
- Improved multicloning sequence with most used restriction sites.
- Potent 3' ribosomal transcriptional terminator.
- Two *Not*I site flanking the promoter fusion for subcloning into integrative mini Tn5 delivery vectors.

The C-LYTAG allows high degree protein purification using cost convenient chromatography supports (C-LYTRAP is more than 5 time less expensive than other commercial resins). This tag also provides improved solubility to recombinant proteins compared to histag and it is compatible with the purification of metalloproteins. The main advantages of the expression and purification systems are:

- Low basal expression level facilitating cloning of toxic proteins and the stability of recombinant strains.
- The CASCADE™ system functions does not require a particular media formulation.
- Active at low temperature of growth.
- Higher level of purification than His-tag.
- Low cost of main consumables (inducer, support, etc) that allows cost efficient scaling up the production.
- Compatibility with mini Tn5 delivery vectors for the construction of stable strains.

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 patented and licensed by Active Motif, Inc. Commercial license available. Please contact us if you want more information about license agreement.

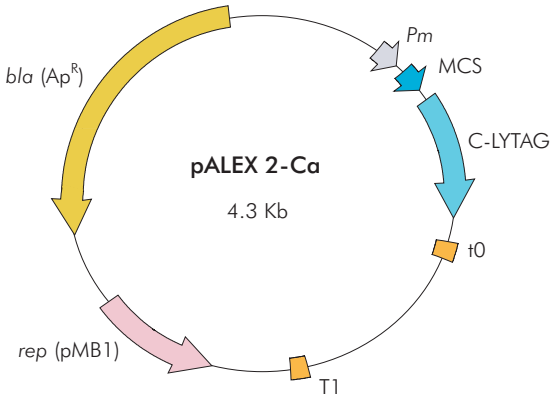
2. Description.

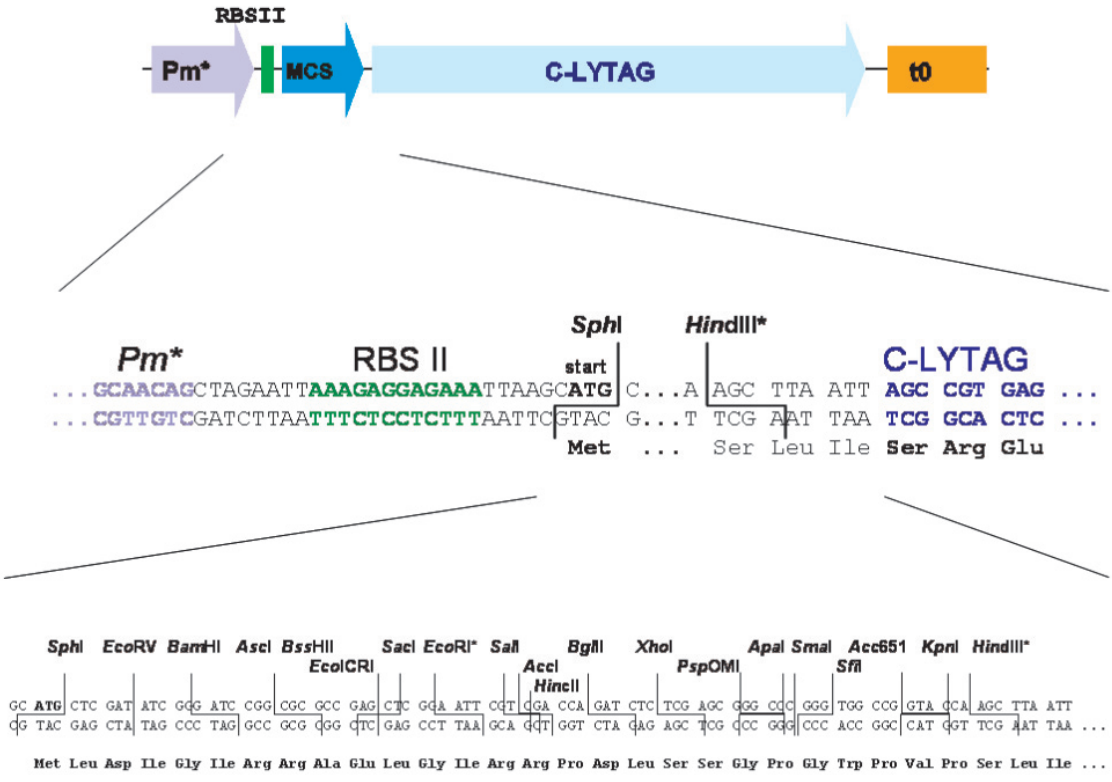
PRODUCT	CAT.No.	FORMAT
pALEX 2-Ca	EV-3435	8 µg

3.1. pALEX2-Ca map and restriction analysis.

pALEX2-Ca vector contains a multiple cloning site (MCS) that has thirteen unique restriction sites: *Sph*I, *Eco*RV, *Bam*HI,*Asc*I,*Sac*I, *Eco*RI, *Sal*I, *Bgl*II, *Xho*I, *Apa*I, *Sma*I, *Sfi*I and *Kpn*I (refer to vector map for details).

pALEX2-Ca and pALEX vectors carry as selection marker the *bla* gene, that confers ampicillin resistance.





(*) *Eco*RI and *Hin*dIII sites shown are not unique in *pALEX2-Ca*

4. Protocol.

4.1. Cloning in to pALEX2-Ca vector 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.
- 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).

* E. coli REG-1 strain contains the regulatory element nahR/Psal::xylS2 integrated into the chromosome.

4.2. 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 (0.05-5 mM salicylate), temperature (16-37°C) and time of induction (1h-overnight).Typical procedures described below:

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 3 hours (until the OD_{600} is 0.7-1).
3. Add the expression inducer (salicylate) to a final concentration of 2 mM.
4. Grow induced culture for 2-5 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.

5. Storage Conditions.

Store at 4°C. For long periods, it is recommended store at -20°C.

For more information,
please visit our WEBSITE
or contact us at

Biomedal S.L.
Av. Americo Vespucio, nº 5-E, pl 1ª
Parque Científico Tecnológico Cartuja 93
41092 - Sevilla - España/Spain
Tel. +34-954081276 Fax. +34-954081279
www.biomedal.com
info@biomedal.com