pALEXa, b & c

(Cat. No. EV-3240, EV-3241, EV-3242 and EV-3413)

For research use only.

1. About BIOMEDAL C-LYTAG System.

The C-LYTAG system integrates advanced technologies developed for the expression and purification of proteins in *E. coli*. Proteins produced with this platform are expressed as translational fusions to the choline binding domain of the *Streptococcus pneumoniae* N-acetylmuramoyl-L-alanine amidase LytA (C-LytA), and purified using a choline analogue affinity resin (C-LYTRAP). C-LYTAG interaction with the C-LYTRAP resin is strong, allowing intensive washing with high salt concentration buffers, and highly pure C-LYTAG-fused proteins can be recovered in a one-step elution process using choline-containing buffers. C-LYTAG – C-LYTRAP interaction is fully reversible, facilitating stable immobilization of purified proteins in this resin, or onto MULTIBIND 96 C-LYTAG microtiter plates (Cat.No. RS-3317/18), for high-throughput analysis purposes. In addition C-LYTAG fusion proteins expressed from pALEX a, b and c incorporate an enterokinase recognition sequence that enables optional removal of the C-LYTAG moiety.

The C-LYTAG system integrates the CASCADE™* technology, a potent and highly regulatable expression system controlled by linked regulatory circuits based on two salicylate-responsive transcriptional activator proteins, NahR and XylS2. In the presence of salicylate, NahR induces expression of XylS2, which in turns activates expression of C-LYTAG translational gene fusions from it cognate promoter, *Pm*, present in the C-LYTAG pALEXa, b and c vectors. The signal-amplifying effect of using two transcriptional regulators in a sequential cascade results in very high expression levels, while maintaining minimal activity in the absence of the inducer.

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

2. Description.

PRODUCT	CAT.No.	FORMAT
pALEX a	EV-3240	8 µg
pALEX b	EV-3241	8 µg
pALEX c	EV-3242	8 µg
pALEX a, b & c	EV-3413	8 µg (each)

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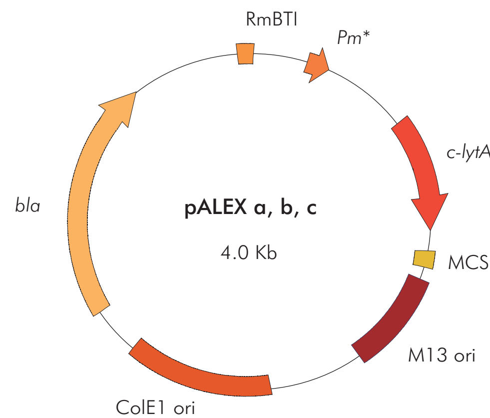
3. pALEX vectors.

3.1. pALEX map.



3.2. General information.

pALEX vectors are ColE1-based plasmids that contain the 3´ region of the *S. pneumoniae lytA* gene encoding the C-LytA protein, located downstream of the *Pm* promoter and followed by a multiple cloning site, with seven unique restriction sites (*Bam*HI, *Xho*I, *Bgl*II, *Kpn*I, *Nde*I, *Bst*BI and *Hin*dIII; refer to vector map for details), in all three reading frames (pALEXa, pALEXb and pALEXc), to facilitate cloning.

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

3.3. Sequence and restriction analysis.

It is indicated:

ORANGE: Pm Promoter
PALE BLUE: Variable region in pALEXa, b and c vectors.
RED: CLYTAG sequence.
BLUE: Enterokinase recognition sequence.
: pALEX-F Primer (Cat. NO. PR-3433)
: pALEX-R Primer (Cat. NO. PR-3434)

pALEXb vector

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                                                    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
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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
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
                                                     BglII
                             EK Clevage Site ▽  BamHI    XhoI  PstI
1151  TGGGTCGGGA TCTGTACGAC GATGACGATA AGGATCCGAG CTCGAGATCT
         G  R  D   L  Y   D   D  D  D   K  D  P  S   S  R  S
      PvuII                   BstBI     NotI
            KpnI  NdeI     EcoRI   HinDIII
1201  GCAGCTGGTA CCATATGGGA ATTCGAAGCT TGCGGCCGCC CAGCTTGCTG
        A  A  G   T  I  W  E   F  E  A   C  G  R   P  A  C  W

1251  GCGTACCGTT CCTGTCTAAA ATCCCTTTAA TCGGCCTCCT GTTTAGCTCC
         R  T  V   P  V    *
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Version A variable region: GATCGATG GGGATCCGAG
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

4. Protocol.

4.1. Cloning in to 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 is right for your project.
- A stop codon must be included to terminate protein translation. pALEX vectors do not include a stop codon.
- 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.com).

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

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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 (0.01 to 2 mM), temperature (22 – 37 °C) and time of induction (4 h – overnight). The following can be a start point expression protocol:

1. Inoculate 5 ml of liquid culture medium, containing 100 μg/ml ampicillin, with a single, freshly transformed colony. Incubate this pre-culture overnight at 37 °C with shaking (225 r.p.m.).

2. Dilute pre-culture 1:100 in fresh, ampicillin-containing medium and continue incubation in the same conditions until OD_{600} reaches 0.7 – 1.0.

3. Add the inducer (salicylate) to a final concentration of 2 mM. Incubate 5 h at 30 °C with shaking (225 r.p.m.).

4. Harvest cells by centrifugation at 4000 xg for 15 min at 4 °C. Disrupt cells for protein extraction and analysis, or store pellet at -20 °C until use.

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

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