

pALEX1 a, b & c

(Cat. No. EV-3439; EV-3440; EV-3441)

For research use only.

1. About BIOMEDAL CASCADE™ System.

The CASCADE™ expression system uses two salicylate-responsive transcriptional activators, NahR and XylS2. In the presence of salicylate, the NahR protein induces expression of XylS2 from the P_{sal} promoter. Salicylate also activates XylS2, and hence its cognate promoter P_m , inducing high-level expression of the gene or genes of interest. The synergistic effect of combining two transcriptional regulators in a sequential cascade amplifies expression levels nearly 20-fold compared to expression from either promoter individually.

2. Description.

PRODUCT	CAT.No.	FORMAT
pALEX 1a	EV-3439	8 µg
pALEX 1b	EV-3440	8 µg
pALEX 1c	EV-3441	8 µg

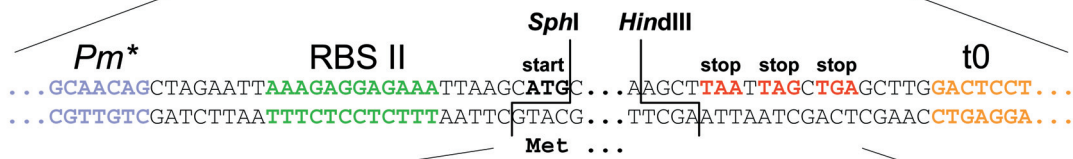
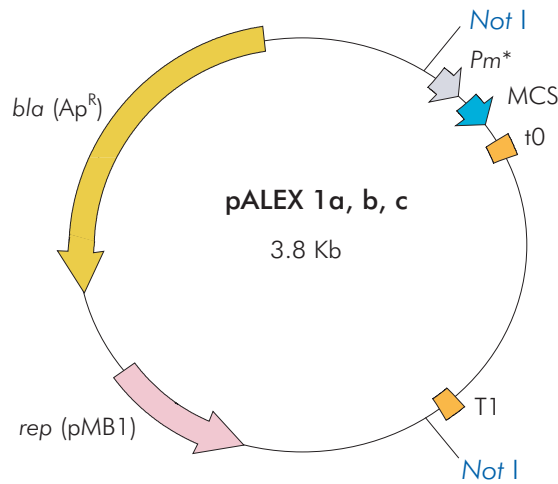
3. pALEX1 vectors.

3.1. General information.

pALEX1a, b and c are highly improved CASCADE™ expression vectors to be used in *E. coli* hosts strains bearing the salicylate-inducible *nahR/Psal::xylS2* regulatory module, such as REG1 or MEDAL-GOLD (Cat. No. BS-3262 and BS-3432). These new vectors are high-copy number, ampicillin-selectable plasmids that incorporate a mutant P_m promoter with even lower basal transcriptional activity, for tightly regulated and high capacity protein expression. Among other features, pALEX1a, b and c have an improved multiple cloning site (MCS), for easy and flexible cloning in-frame with an ATG start codon located at an optimal distance from a highly efficient ribosome binding site (RBSII), as well as stop codons in all three forward reading frames, followed by strong transcriptional terminator sequences (t0 and T1). In addition, the expression cassette in pALEX1a, b and c is flanked by "rare cutter" *NotI* sites, for convenient subcloning into mini Tn5 delivery vectors and construction of genetically stable expression strains.

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3.2. pALEX1a, 1b and 1c maps.



pALEX1a

SphI **EcoRV** **BamHI** **Ascl** **BssHII** **SacI** **EcoRI** **Sall** **BglII** **XhoI** **Apal** **SmaI** **Acc651** **KpnI** **HindIII**

EcoICRI **AccI** **HincII** **PspOMI** **SfiI**

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GC ATG CTC GAT ATC GGG ATC CGG CGC GCC GAG CTC GGA ATT CGT CGA CCA GAT CTC TCG AGC GGG CCC GGG TGG CCG GTA CCA AGC TTA ATT AGC TGA
CG TAC GAG CTA TAG CCC TAG GCC GCG CCG CTC GAG CCT TAA GCA GGT GGT CTA GAG AGC TCG CCC GGG CCC ACC GGC CAT GST TCG AAT TAA TCG ACT
Met Leu Asp Ile Gly Ile Arg Arg Ala Glu Leu Gly Ile Arg Arg Pro Asp Leu Ser Ser Gly Pro Gly Trp Pro Val Pro Ser Leu Ile Ser ---
    
```

pALEX1b

SphI **EcoRV** **BamHI** **Ascl** **BssHII** **SacI** **EcoRI** **Sall** **BglII** **XhoI** **Apal** **SmaI** **Acc651** **KpnI** **HindIII**

EcoICRI **AccI** **HincII** **PspOMI** **SfiI**

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GC ATG CTC TCG ATA TCG GGA TCC GGC GCG CCG AGC TCG GAA TTC CTC GAC CAG ATC TCT CGA GCG GGC CCG GGT GGC CCG TAC CAA GCT TAA TTA GCT GA
CG TAC GAG AGC TAT AGC CCT AGG CCG CGC GGC TCG AGC CTT AAG CAG CCG GTC TAG AGA GCT CCG CCC GGC CCA CCG GGC ATG GTT CGA ATT AAT CGA CT
Met Leu Ser Ile Ser Gly Ser Gly Ala Pro Ser Ser Glu Phe Val Asp Gln Ile Ser Arg Ala Gly Pro Gly Gly Arg Tyr Gln Ala ---
    
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pALEX1c

SphI **EcoRV** **BamHI** **Ascl** **BssHII** **SacI** **EcoRI** **Sall** **BglII** **XhoI** **Apal** **SmaI** **Acc651** **KpnI** **HindIII**

EcoICRI **AccI** **HincII** **PspOMI** **SfiI**

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GC ATG CTT CGA TAT CGG GAT CCG GCG GCG CGA GCT CCG AAT TCG TCG ACC AGA TCT CTC GAG CCG GCC CCG GFG GCC GGT ACC AAG CTT AAT TAG CTG A
CG TAC GAA GCT ATA GCC CTA GGC CGC GCG GGT CGA GCC TTA AGC AGC TGG TCT AGA GAG CTC GCC CCG GCC CAC CCG CCA TGG TTC GAA TTA ATC GAC CT
Met Leu Arg Tyr Arg Asp Pro Ala Arg Arg Ala Arg Asn Ser Ser Thr Arg Ser Leu Glu Arg Ala Arg Val Ala Gly Thr Lys Leu Asn ---
    
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*Variable region in pALEX1b and pALEX1c vectors

4. Protocol.

4.1. Cloning into pALEX1 vectors and host strain transformation.

To facilitate cloning strategies, the MCSs of pALEX1a, b and c contain unique recognition sites for a wide variety of restriction enzymes. Assembly of a construct leading to expression of the desired protein will require using the appropriate restriction enzymes, to insert the coding sequence in-frame with the ATG start codon in the MCS. The pALEX1a, b and c vectors differ in one, or two base-pair insertions that introduce frame shifts in the MCS region located between the *SphI* and *EcoRV* sites, simplifying in-frame cloning by choosing the appropriate pALEX1 vector. Expression of the resulting protein can be carried out in one of the CASCADE™ bacterial hosts (*E. coli* REG-1, Cat. No. BS-3262 or *E. coli* MEDAL GOLD, Cat. No. BS-3432). Biomedal recommends TSS solution (Cat. No. RS-3215/16), as a fast and simple method to prepare transformation-competent bacteria (for more information, visit our website at www.biomedal.com)

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 (200 r.p.m.).
2. Dilute pre-culture 1:100 in fresh, ampicillin-containing medium and continue incubation in the same conditions until OD₆₀₀ 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 (200 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,
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or contact us at

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