evoglow®

basic kit

product information

evocatal

Cat. No.: 2.1.010

Flavin-mononucleotide-based Fluorescent Protein (FbFP)

evoglow® basic kit

Catalog No. evo-2.1.010 **Quantity 20 µg each**

General Information

Fluorescent reporter proteins are valuable noninvasive molecular tools for *in vivo* real-time imaging of living cells and tissues as well as *in vitro* fluorescence labeling. A major drawback of existing GFP-like reporter proteins is their strict requirement for molecular oxygen as a cofactor for the synthesis of their respective chromophores. Therefore, the application in anaerobic systems is not possible (see Fig. 1).

The flavin mononucleotide (FMN)-based fluorescent proteins (FbFPs) from the evoglow® series were developed to overcome these restrictions. The evoglow® proteins can be used as fluorescent reporters in both aerobic and anaerobic biological systems.

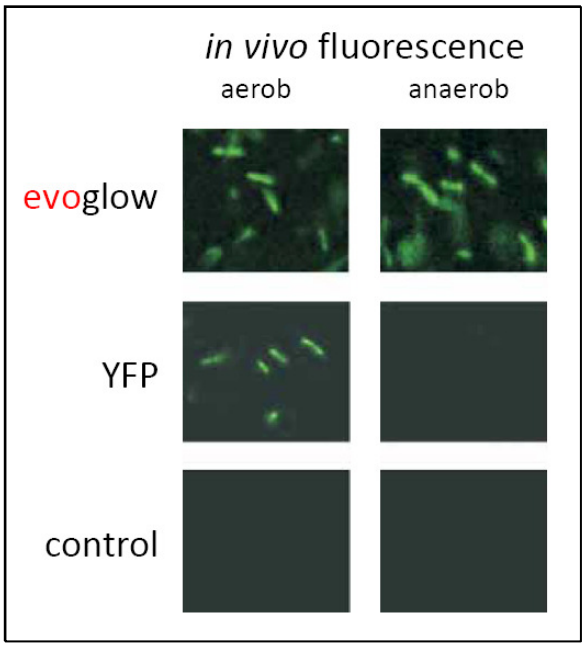


Fig. 1: *in vivo*-fluorescence of **evoglow**®-proteins compared to that of conventional Yellow Fluorescent Protein (YFP).

Kit content: The evoglow® basic kit contains the plasmids pGLOW-Bs1, pGLOW-Bs1-stop, pGLOW-Bs2, pGLOW-Bs2-stop, pGLOW-Pp1 and pGLOW-Pp1-stop. The genes are cloned *Sac*I/*Pst*I or *Sac*I/*Bam*HI into the multiple cloning site of pUC18 for individual subcloning. Each gene is available in a fusion-version - without stop codon - and in an expression-version containing the TGA stop codon.

Origin: The evoglow-Bs1 and Bs2 genes were cloned from the Gram positive bacterium *Bacillus subtilis*. The Bs1-sequence is the wild-type codon-usage, whereas Bs2-sequence has been adjusted to *Escherichia coli* codon usage and is truncated, i.e. comprises the LOV-domain only. The evoglow-Pp1 gene was cloned from the Gram negative bacterium *Pseudomonas putida*.

Formulation: pGLOW plasmids are delivered as 20 µg plasmid DNA dissolved in regular 10 mM Tris/HCl-buffer, pH 8,5.

Storage: pGLOW plasmids can be stored at -20°C.

Intended use: For research use only. Not for clinical diagnosis.

References: Drepper, T. *et al.* (2007) *Nat. Biotechnol.* 25: 443-445.

evoglow®- Anaerobic Fluorescent Proteins

The designation evoglow® comprises a novel type of fluorescent proteins containing a Flavin-mono-nucleotide-based cofactor. They are thus capable of developing bright cyan-green fluorescence even in the complete absence of oxygen. They fold rapidly *in vivo* and thus enable immediate detection of expression to study anaerobic inter- and intracellular processes. Since their fluorescence intensity as well as the excitation and emission spectra of the *evoglow*® proteins lie within the same range as those of other fluorescent proteins, the same instrumental setup can be used for their detection and analysis as for other fluorescent proteins. Recommendations for instruments and filtersets are given in section *Instrumental Setup*.

Physical Parameters:

Characteristics	evoglow®-Bs1	evoglow®-Bs2	evoglow®-Pp1
Fluorescence color	cyan-green	cyan-green	cyan-green
Excitation max. (nm)	450	450	450
Emmission max. (nm)	495	495	495
Quantum yield	0,4	0,39	0,17
Extinction coefficient ($M^{-1}cm^{-1}$)	12,500	12,500	12,500
Brightness*	5	4.9	2.1
pKa	n.d.	n.d.	n.d.
structure	monomer	dimer	dimer
Photostability	+	++	+++
Molecular weight	ca. 33kDa	ca. 19kDa	ca. 19kDa
Amino acids	262	137	148

Instrumental Setup Recommendations

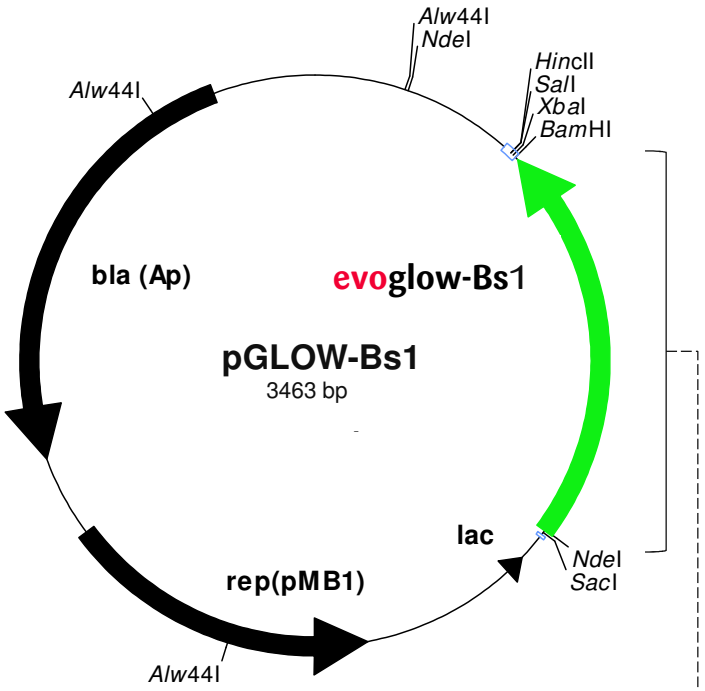
evoglow® proteins can be excellently detected by using the following instrumental setup:

- microscope: Zeiss Axioplan 1.
- object lens for fluorescence imaging: Plan APOCHROMAT 100x Öl DIC mit numerical aperature 1,4 (object lens "Plan-Apochromat" 100x/1,40 Oil DIC) (Immersion oil 518F)
- filterset 37 (Ex 450/50, BS 480, Em 510/50)
- lamp: HBO50
- camera: Zeiss AxioCam MRm (12 Bit, monochrome)
- standard imaging software: AxioVision Rel. 4.6.
- for light microscopy: Zeiss Plan NEOFLUAR 100x Öl phase contrast objective (Phase 3) numerical aperature 1,3 (object lens "Plan-Neofluar" 100x/1,30 Oil Ph3)

pGLOW-Bs1 and pGLOW-Bs1-stop

Plasmid Information

Plasmid Map



restriction analysis information:

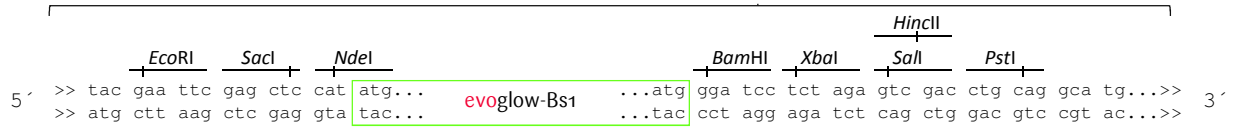
enzyme:	fragment size (bp)
*Alw*44I	497
	1246
	1720

further restriction sites at positions (bp)

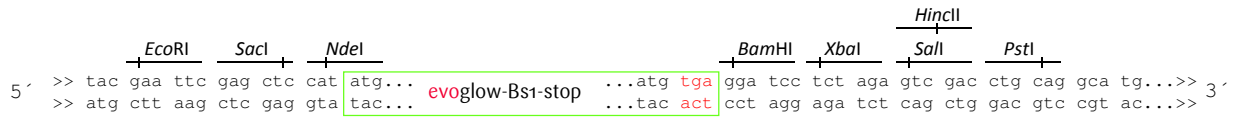
*Eco*RI	851	1227
*Hind*III	399	813
*Nde*I	183	1215
*Pst*I	411	998

sequence landmarks (bp positions):

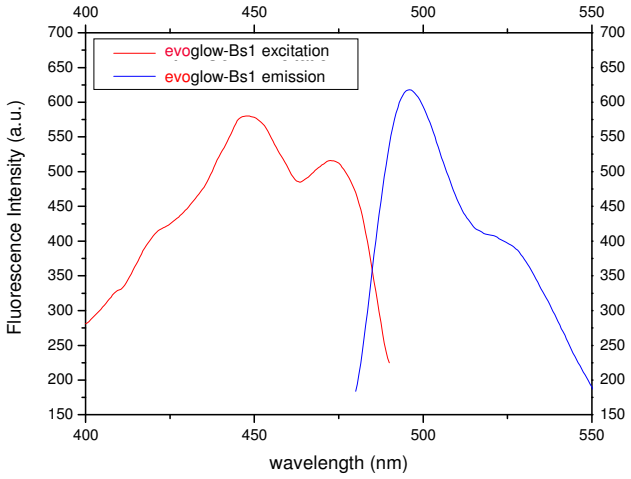
ampicillin-resistence:	3263–2403
rep (pMB1):	2243–1629
lac-promotor:	1323– 284
evoglow-Bs1-gene:	1217– 435



In pGLOW-Bs1-stop the evoglow-Bs1-gene includes a termination codon indicated in red



Fluorescence Spectra:



Technical Data:

excitation max.:	449 nm
emission max.:	495 nm (λ_{ex}: 450 nm)
molar extinction coefficient (FMN):	ε = 12,500 ±500 M^{-1} cm^{-1} (at 450 nm)
quantum yield:	Q_F = 0,38

Sequence Information:

evoglow-Bs1 nucleotide sequence (786 bp)

```
  1 ATGGCTAGTT  TTCAATCATT  TGGGATACCA
 31 GGACAGCTGG  AAGTCATCAA  AAAAGCACTT
 61 GATCACGTGC  GAGTCGGTGT  GGTAATTACA
 91 GATCCCGCAC  TTGAAGATAA  TCCTATTGTC
121 TACGTAAATC  AAGGCTTTGT  TCAAATGACC
151 GGCTACGAGA  CCGAGGAAAT  TTTAGGAAAG
181 AACGCACGCT  TCTTACAGGG  GAAACACACA
211 GATCCTGCAG  AAGTGGACAA  CATCAGAACC
241 GCTTTACAAA  ATAAAGAACC  GGTCACCGTT
271 CAGATCCAAA  ACTACAAAAA  AGACGGAACG
301 ATGTTCTGGA  ATGAATTAAA  TATTGATCCA
331 ATGGAAATAG  AGGATAAAAC  GTATTTTGTC
361 GGAATTCAGA  ATGATATCAC  CAAGCAAAAA
391 GAATATGAAA  AGCTTCTCGA  GGATTCCCTC
421 ACGGAAATTA  CTGCACTTTC  AACTCCTATT
451 GTCCCGATTC  GCAATGGCAT  TTCGGCTCTT
481 CCGCTAGTCG  GAAACCTGAC  AGAGGAGCGA
511 TTTAATTCCA  TCGTTTGCAC  ATTGACGAAT
541 ATCTTATCAA  CATCCAAAGA  TGATTATTTG
571 ATCATTGATT  TATCCGGATT  GGCCCAAGTG
601 AACGAACAAA  CGGCCGACCA  AATTTTCAAG
631 CTGAGCCATT  TGCTGAAATT  GACCGGAACT
661 GAGTTAATCA  TTACTGGCAT  TAAGCCTGAA
691 TTGGCTATGA  AAATGAATAA  ACTGGATGCC
721 AATTTTTCGT  CGCTGAAAAC  ATATTCAAAT
751 GTAAAGGATG  CCGTTAAAGT  GCTTCCGATT
781 ATG TGA  term. codon in pGLOW-Bs1-stop only
```

evoglow-Bs1 amino acid sequence (261 aa)

```
  1 MASFQSFGIP  GQLEVIKKAL  DHVRVGVVIT
 31 DPALEDNPIV  YVNQGFVQMT  GYETEEILGK
 61 NARFLQGKHT  DPAEVDNIRT  ALQNKEPVTV
 91 QIQNYKKDGT  MFWNELNIDP  MEIEDKTYFV
121 GIQNDITKQK  EYEKLLEDSL  TEITALSTPI
151 VPIRNGISAL  PLVGNLTEER  FNSIVCTLTN
181 ILSTSKDDYL  IIDLSGLAQV  NEQTADQIFK
211 LSHLLKLTGT  ELIITGIKPE  LAMKMNKLDA
241 NFSSLKTYSN  VKDAVKVLPI  M
```

Note:

The nucleotide sequence of evoglow-Bs1-stop is identical to that of evoglow-Bs1 but includes a termination-codon at the 3'-end as indicated in the adjacent sequence.
The plasmid base pair numbering is hence shifted 3 positions in the 'stop'-constructs.

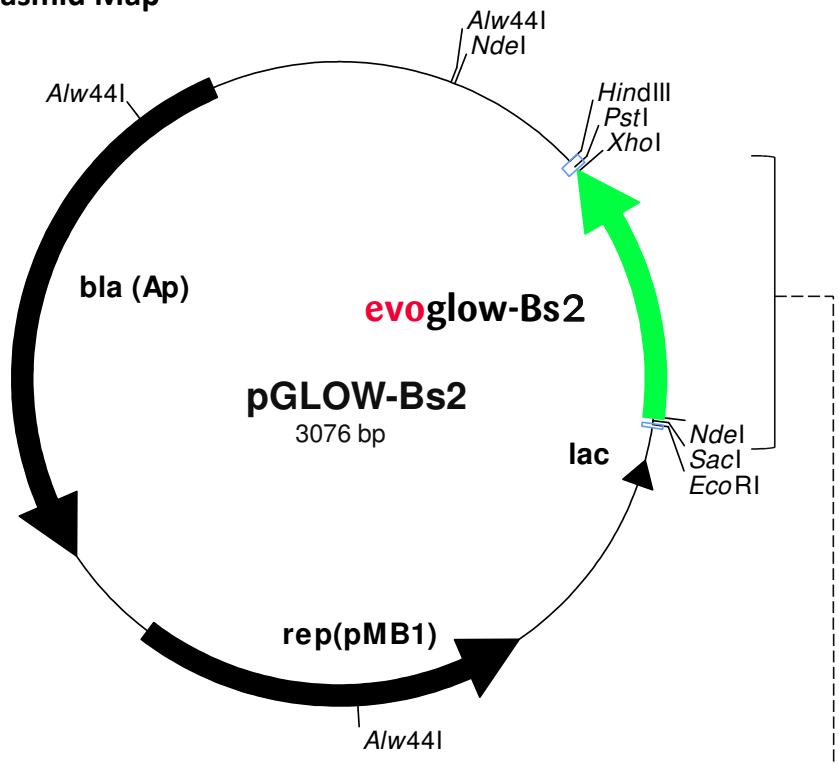
All plasmids in this kit are derived from pUC18 and replicate only in *E. coli* and closely related microorganisms.

Plasmids are not suitable for expression.

pGLOW-Bs2 and pGLOW-Bs2-stop

Plasmid Information

Plasmid Map



restriction analysis information:

enzyme	fragment size (bp)
*Alw*44I	497
	1246
	1331

further restriction sites at (bp)

*Eco*RI		851
*Hind*III		399
*Nde*I	183	828
*Pst*I		411
*Sac*I		834
*Xho*I		420

sequence landmarks (bp):

ampicillin-esistence:	2876–2016
rep (pMB1):	1856–1242
lac-promotor:	936– 897
evoglow-Bs2-gene:	830– 417

*EcoR*I *Sac*I *Nde*I *Pst*I *Sph*I *Hind*III

5´ >> tac gaa ttc gag ctc cat atg... evoglow-Bs2 ...gag gct gca ggc atg caa gct tgg...>> 3´

>> atg ctt aag ctc gag gta tac... ...ctc gga cgt ccg tac gtt cga acc...>>

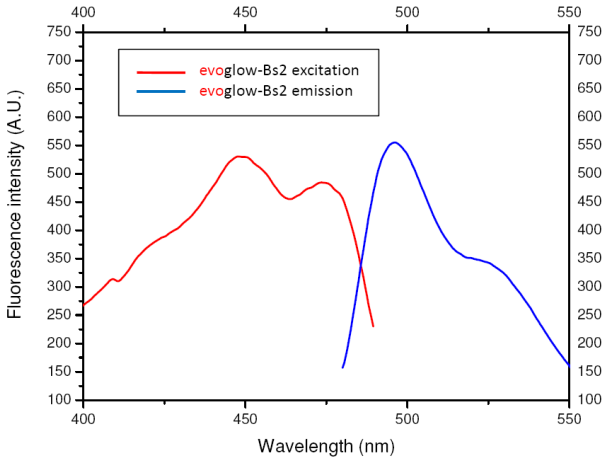
In pGLOW-Bs2-stop the evoglow-Bs2-gene includes a termination codon indicated in red

*EcoR*I *Sac*I *Nde*I *Pst*I *Sph*I *Hind*III

5´ >> tac gaa ttc gag ctc cat atg... evoglow-Bs2 ...gag tga ctg cag gca tgc aag ctt ggc...>> 3´

>> atg ctt aag ctc gag gta tac... ...ctc act gac gtc cgt acg ttc gaa ccg...>>

Fluorescence Spectra:



Technical Data:

excitation max.:	449 nm
emission max.:	495 nm (λ_{ex}: 450 nm)
molar extinction coefficient (FMN):	ε = 12,500 ±500 M^{-1} cm^{-1} (at 450 nm)
quantum yield:	Q_F = 0,39

Sequence Information:

evoglow-Bs2 nucleotide sequence (411 bp)

```
  1 ATGGCGTCGT  TCCAGTCGTT  CGGCATCCCG
 31 GGCCAGCTGG  AAGTCATCAA  GAAGGCGCTG
 61 GATCACGTGC  GCGTCGGCGT  GGTCATCACC
 91 GATCCCGCGC  TGGAAGATAA  CCCGATCGTC
121 TACGTGAACC  AGGGCTTCGT  GCAGATGACC
151 GGCTACGAGA  CCGAGGAAAT  CCTGGGCAAG
181 AACGCGCGCT  TCCTCCAGGG  GAAGCACACC
211 GATCCGGCGG  AAGTGGACAA  CATCCGCACC
241 GCGCTGCAAA  ATAAAGAACC  GGTCACCGTG
271 CAGATCCAGA  ACTACAAGAA  GGACGGCACG
301 ATGTTCTGGA  ACGAACTGAA  CATCGATCCG
331 ATGGAAATCG  AGGATAAGAC  GTATTTCGTC
361 GGCATCCAGA  ACGACATCAC  CAAGCAGAAG
391 GAATATGAAA  AGCTGCTCGA  G TGA
```

termination codon in pGLOW-Bs2-stop only

evoglow-Bs2 amino acid sequence (137 aa)

```
  1 MASFQSFGIP  GQLEVIKKAL  DHVRVGVVIT
 31 DPALEDNPIV  YVNQGFVQMT  GYETEEILGK
 61 NARFLQGKHT  DPAEVDNIRT  ALQNKEPVTV
 91 QIQNYKKDGT  MFWNELNIDP  MEIEDKTYFV
121 GIQNDITKQK  EYEKLLE
```

Note:

The nucleotide sequence of evoglow-Bs2-stop is identical to that of evoglow-Bs2 but includes a termination-codon at the 3'-end as indicated in red in the adjacent sequence. The plasmid base pair numbering is hence shifted 3 positions in the 'stop'-constructs.

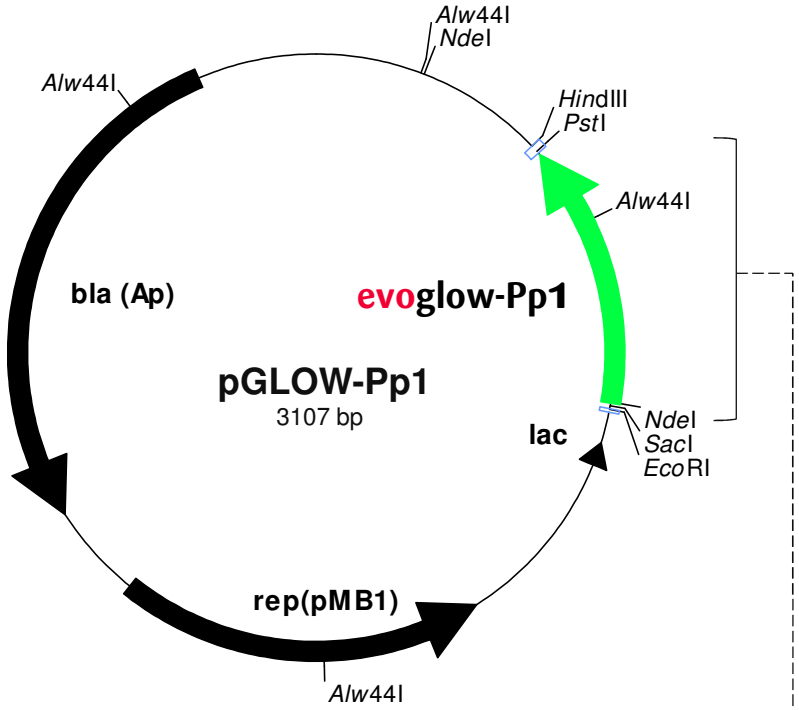
All plasmids in this kit are derived from pUC18 and replicate only in *E. coli* and closely related microorganisms.

Plasmids are not suitable for expression.

pGLOW-Pp1 and pGLOW-Pp1-stop

Plasmid Information

Plasmid Map



restriction analysis information:

enzyme:	fragment size (bp)
*Alw*44I	376
	497
	988
	1246

further restriction sites at (bp)

*Eco*RI		871
*Hind*III		399
*Nde*I	183	859
*Pst*I		411
*Sac*I		865

sequence landmarks (bp positions):

ampicillin-resistence:	2907–2047
rep (pMB1):	1887–1273
lac-promotor:	967– 928
evoglow-Pp1-gene:	861– 418

*EcoR*I *Sac*I *Nde*I *Pst*I *Sph*I *Hind*III

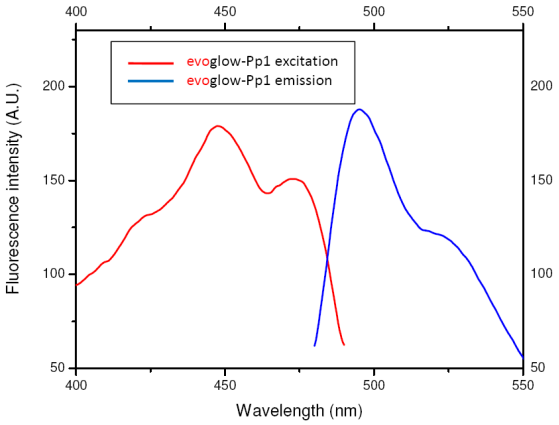
5´ >> tac gaa ttc gag ctc cat atg... evoglow-Pp1 ...cac gct gca ggc atg caa gct tgg c...>> 3´
>> atg ctt aag ctc gag gta tac... ...gtg cga cgt ccg tac gtt cga acc g...>>

In pGLOW-Bs2-stop the evoglow-Bs2-gene includes a termination codon indicated in red

*EcoR*I *Sac*I *Nde*I *Pst*I *Sph*I *Hind*III

5´ >> tac gaa ttc gag ctc cat atg... evoglow-Pp1 ...cac tga ctg cag gca tgc aag ctt ggc...>> 3´
>> atg ctt aag ctc gag gta tac... ...gtg act gac gtc cgt acg ttc gaa ccg...>>

Fluorescence Spectra:



Technical Data:

excitation max.:	449 nm
emission max.:	495 nm (λ_{ex}: 450 nm)
molar extinction coefficient (FMN):	ε = 12,500 ±500 M^{-1} cm^{-1} (at 450 nm)
quantum yield:	Q_F = 0,17

Sequence Information:

evoglow-Pp1 nucleotide sequence (444 bp)

```
  1 ATGATCAACG  CAAAACTCCT  GCAACTGATG
 31 GTCGAACATT  CCAACGATGG  CATCGTTGTC
 61 GCCGAGCAGG  AAGGCAATGA  GAGCATCCTT
 91 ATCTACGTCA  ACCCGGCCTT  CGAGCGCCTG
121 ACCGGCTACT  GCGCCGACGA  TATTCTCTAT
151 CAGGACGCCC  GTTTTCTTCA  GGGCGAGGAT
181 CACGACCAGC  CGGGCATCGC  AATTATCCGC
211 GAGGCGATCC  GCGAAGGCCG  CCCCTGCTGC
241 CAGGTGCTGC  GCAACTACCG  CAAAGACGGC
271 AGCCTGTTCT  GGAACGAGTT  GTCCATCACA
301 CCGGTGCACA  ACGAGGCGGA  CCAGCTGACC
331 TACTACATCG  GCATCCAGCG  CGATGTCACA
361 GCGCAAGTAT  TCGCCGAGGA  AAGGGTTCGC
391 GAGCTGGAGG  CTGAAGTGGC  GGAACTGCGC
421 CGGCAGCAGG  GCCAGGCCAA  GCAC TGA
```

termination codon in pGLOW-Pp1-stop only

evoglow-Pp1 amino acid sequence (148 aa)

```
  1 MINAKLLQLM  VEHSNDGIVV  AEQEGNESIL
 31 IYVNPAFERL  TGYCADDILY  QDARFLQGED
 61 HDQPGIAIIR  EAIREGRPCC  QVLRNYRKDG
 91 SLFWNELSIT  PVHNEADQLT  YYIGIQRDVT
121 AQVFAEERVR  ELEAEVAELR  RQQGQAKH
```

Note:

The nucleotide sequence of evoglow-Pp1-stop is identical to that of evoglow-Pp1 but includes a termination-codon at the 3'-end as indicated in red in the adjacent sequence. The plasmid base pair numbering is hence shifted 3 positions in the 'stop'-constructs.

All plasmids in this kit are derived from pUC18 and replicate only in *E. coli* and closely related microorganisms.

Plasmids are not suitable for expression.

Contact Information:

For any further information, questions or remarks please contact us:

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