- express^{N} kit –

broad host range vectors

- gram negative bacteria -

product information

evocatal

Cat. No.: 2.1.020

Content:

Product Overview

evoglow® express^{N}-kit

The evoglow® express^{N}-kit contains the entire collections of the pGLOW^{XN}-plasmids, a plasmid set designed for the efficient expression of the evoglow® fluorescent reporter proteins. These proteins are a novel type of flavin-mononucleotide based proteins (FbFP´s) which are fluorescent under both aerobic and anaerobic conditions. Key feature of the evoglow® proteins is their Flavinmononucleotide-based cofactor that does not require any oxygen for its biosynthesis. The protein is rapidly expressed and fully functional even in strict anaerobic environments *in vivo (see references).*

All plasmids of the pGLOW^{XN}-series contain genes coding for one of three available evoglow® fluorescent proteins enabling rapid detection of your target cell structure or organism. Choose between a consitutive promotor for continuous expression or an inducible T7-promotor for the strong induction of fluorescence at a distinct point of time. The pGLOW^{XN} vectors are designed for use in a broad host range of gram negative host organisms like *E.coli*, *Pseudomonas spp.*, *Rhodobacter spp. and many more*. Choose the best vector of the pGLOW^{XN}- series to and cast bright light onto your research project!

The evoglow®-Fluorescent Proteins

The designation evoglow® comprises a novel type of fluorescent proteins containing a Flavin-mononucleotide-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*.

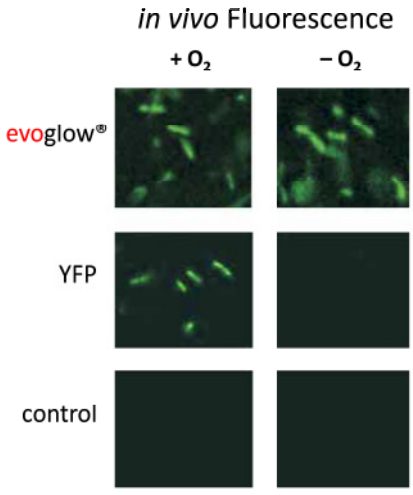


Abb. 1: Comparison of fluorescence-performance in vivo of evoglow® and common YFP under aerobic and anaerobic conditions

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

Plasmid Information

The pGLOW^{XN}-Plasmid are designed for the specific and efficient expression of evoglow® fluorescent proteins. Two types of pGLOW^{XN}-vectors are available (see Tab. 1):

pGLOW-T^{XN} contains an inducible promoter (PT7) for the rapid and strong induction of expression. pGLOW-K^{XN} contains the consitutive promoter aphII as well as PT7 and thus enables monitoring of continuous expression levels as well as the enhancement of expression levels at a given point of time.

Both plasmids can be selected for by using chloramphenicol and/or kanamycin at concentrations depending on the specific host organism used. They also contain *rep*-genes for replication in a broad host range of gram-negative microorganisms rendering them compatible with other plasmids e.g. of the ColE1- or p15A-type. Furthermore *mob*-genes are present to enable plasmid transfer by conjugation.

Tab. 1: Overview of the pGLOW-vector features. For sequence details see plasmid maps, page 9 ff.

vector features:	**pGLOW-T^{XN}**	**pGLOW-K^{XN}**
Vector Type	bacterial expression vector	bacterial expression vector
Promotor	PT7	PaphII, PT7
Inducer	IPTG	IPTG
Antibiotic Resistance	chloramphenicol, kanamycin	chloramphenicol, kanamycin
Host Range / Host Org.	prokaryotic, gram-negative	prokaryotic, gram-negative
rep	Gene for plasmid replication in a broad range gram-negative bacteria; compatible with ColE1, p15A and most common origins of replication	
mob	required for plasmid mobilization; codes for a relaxase binding specifically to the transfer origin (RSA) in order to nick the DNA and regulate its own synthesis.	
Comments	PT7 is a strong inducible promotor for efficient overexpression of the GOI in strains harbouring the T7-polymerase. Expression is induced by addition of low concentrations of IPTG (see protocol section).	aphII is a constitutive promotor for continious expression of the GOI. The plasmid contains pT7 as well and thus efficient overexpression can be induced by addition of low concentrations of IPTG in T7-polymerase-harbouring host strains (see protocol section).

Delivery Notes and Storage Information

All pGLOW^{XN}- plasmids are supplied as purified plasmid DNA in quantities of 20 µg each. Store plasmids at -20°C in the dark. Avoid frequent cycles of thawing and freezing. Aliquot plasmid solutions into smaller volumes.

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)

evoglow express^{N}-kits and pGLOW^{XN}-vectors available:

product	Cat. No.	content	academic price (€)	industry price (€)
pGLOW-T^{XN}-Bs1	2.1.023	1 plasmid	450,00	900,00
pGLOW-T^{XN}-Bs2	2.1.024	1 plasmid	450,00	900,00
pGLOW-T^{XN}-Pp1	2.1.025	1 plasmid	450,00	900,00
pGLOW-K^{XN}-Bs1	2.1.026	1 plasmid	450,00	900,00
pGLOW-K^{XN}-Bs2	2.1.027	1 plasmid	450,00	900,00
pGLOW-K^{XN}-Pp1	2.1.028	1 plasmid	450,00	900,00
evoglow express^{KN} kit	2.1.022	pGLOW-K^{XN}-plasmids combined as a kit (3 plasmids)	1250,00	2500,00
evoglow express^{TN} kit	2.1.021	pGLOW-T^{XN}-plasmids combined as a kit (3 plasmids)	1250,00	2500,00
evoglow express^{N} kit	2.1.020	pGLOW-T^{XN}- plasmids and pGLOW-K^{XN}-plasmids combined as a kit (6 plasmids)	2500,00	5000,00

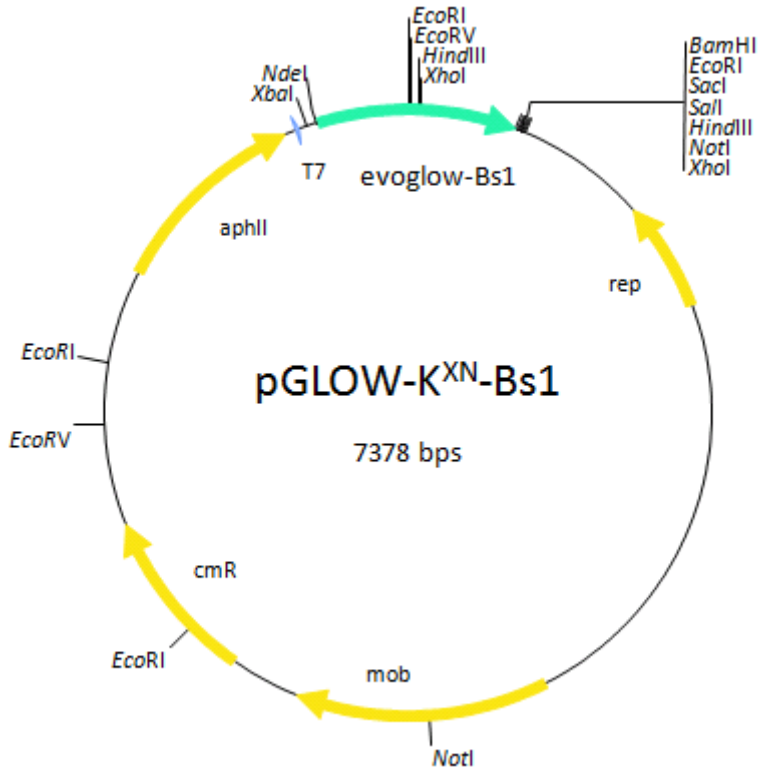
For further information please contact **evocatal** or **BioCat** under adresses given at the end of this manual.

References:

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

Plasmid Maps:

pGLOW-K^{XN}-Bs1



restriction analysis information:

enzyme	bp position			
*Bam*HI	469			
*Eco*RI	04	435	4620	5729
*Eco*RV	15	5488		
*Hind*III	42	454		
*Nde*I	7018			
Not	460	3609		
*Sac*I	441			
*Sal*I	448			
*Xba*I	6979			
*Xho*I	48	469		

sequence landmarks:

marker	bp-positions	
rep	1418-	991
mob	3135-	4128
cmR	4407-	5087
PaphII	6094-	6888
PT7	6933-	6948

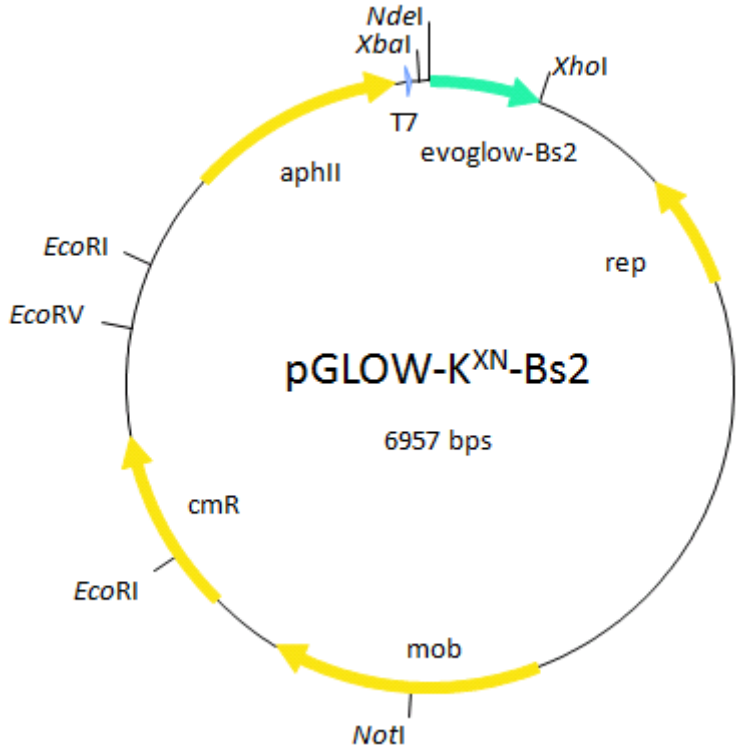
XbaI *NdeI* *BamHI*

```
CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG GCT . . . evoglow-Bs1 . . ATG TAA GGA TCC
GGG AGA TCT TTA TTA AAA CAA ATT GAA ATT CTT CCT CTA TAT GTA TAC CGA . . .                TAC ATT CCT AGG
```

EcoRI *SacI* *SalI* *HindIII* *NotI* *XhoI*

```
GAA TTC GAG CTC CGT CGA CAA GCT TGC GGC CGC ACT CGA GCA CCA CCA CCA CCA CCA CTG TAC ATT CCT AGA TCC
CTT AAG CTC GAG GCA GCT GTT CGA ACG CCG GCG TGA GCT CGT GGT GGT GGT GGT GGT GAC ATG TAA GGA TCT AGG
```

pGLOW-K^{XN}-Bs2



restriction analysis information:

enzyme	bp position	
*Eco*RI	4558	5667
*Eco*RV	5426	
*Nde*I	6956	
*Xba*I	6917	
*Xho*I	407	

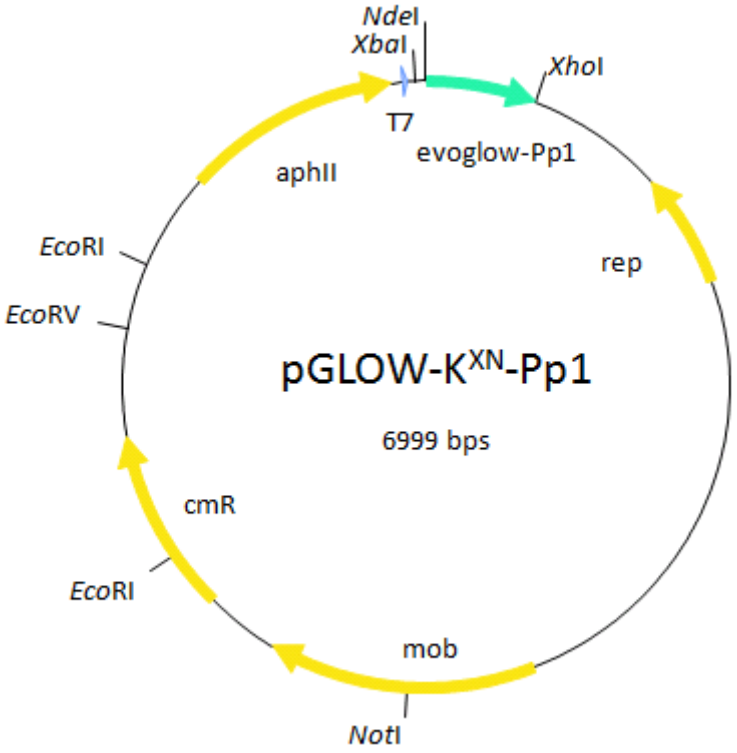
sequence landmarks:

marker	bp-positions	
rep	1356-	929
mob	3073-	4066
cm^R	4345-	5025
PaphII	6032-	6826
PT7	6871-	6886

XbaI *NdeI* *XhoI*

```
CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG GCT... evoglow-Bs2 ...TGC TCG AGC ACC
GGG AGA TCT TTA TTA AAA CAA ATT GAA ATT CTT CCT CTA TAT GTA TAC CGA...                ...ACG AGC TCG TGG
```

pGLOW-K^{XN}-Pp1



restriction analysis information:

enzyme	bp position	
*Eco*RI	4558	5667
*Eco*RV	5426	
*Nde*I	6956	
*Xba*I	6917	
*Xho*I	407	

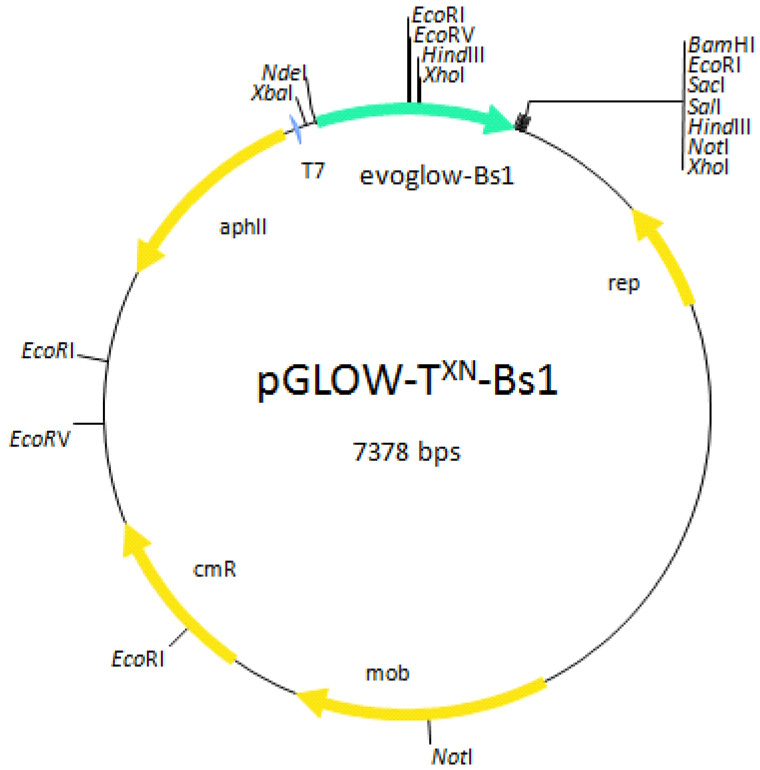
sequence landmarks:

marker	bp-positions	
rep	1398-	971
mob	3115-	4108
cm^{R}	4387-	5067
PaphII	6074-	6868
PT7	6871-	6886

XbaI *NdeI* *XhoI*

```
CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG GCT... evoglow-Pp1 ...CTC GAG CAC CAC
GGG AGA TCT TTA TTA AAA CAA ATT GAA ATT CTT CCT CTA TAT GTA TAC CGA...                ...GAG CTC GTG GTG
```

pGLOW-T^{XN}-Bs1



restriction analysis information:

enzyme	bp position			
*Bam*HI	469			
*Eco*RI	04	435	4620	5729
*Eco*RV	15	5488		
*Hind*III	42	454		
*Nde*I	7018			
*Not*I	460	3609		
*Sac*I	441			
*Sal*I	448			
*Xba*I	6979			
*Xho*I	48	469		

sequence landmarks:

marker	bp-positions	
rep	1418-	991
mob	3135-	4128
cm^R	4407-	5087
PaphII	6549-	5755
PT7	6933-	6948

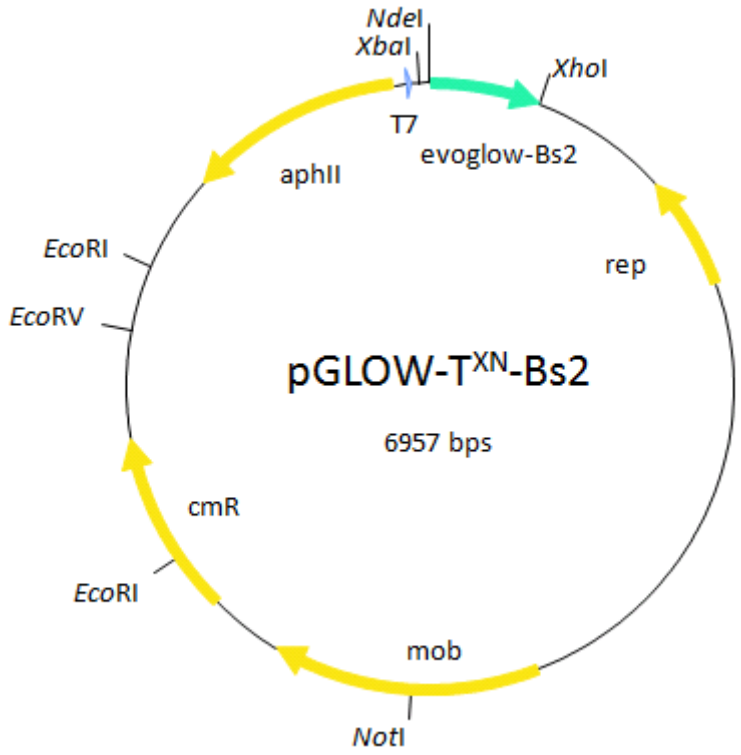
*Xba*I … *Nde*I … *Bam*HI

```
CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG GCT . . . evoglow-Bs1 . . . ATG TAA GGA TCC
GGG AGA TCT TTA TTA AAA CAA ATT GAA ATT CTT CCT CTA TAT GTA TAC CGA . . .             . . . TAC ATT CCT AGG
```

*Eco*RI … *Sac*I … *Sal*I … *Hind*III … *Not*I … *Xho*I

```
GAA TTC GAG CTC CGT CGA CAA GCT TGC GGC CGC ACT CGA GCA CCA CCA CCA CCA CCA CTG TAC ATT CCT AGA TCC
CTT AAG CTC GAG GCA GCT GTT CGA ACG CCG GCG TGA GCT CGT GGT GGT GGT GGT GGT GAC ATG TAA GGA TCT AGG
```

pGLOW-T^{XN}-Bs2



restriction analysis information:

enzyme	bp position	
*Eco*RI	4558	5667
*Eco*RV	5426	
*Nde*I	6956	
*Xba*I	6917	
*Xho*I	407	

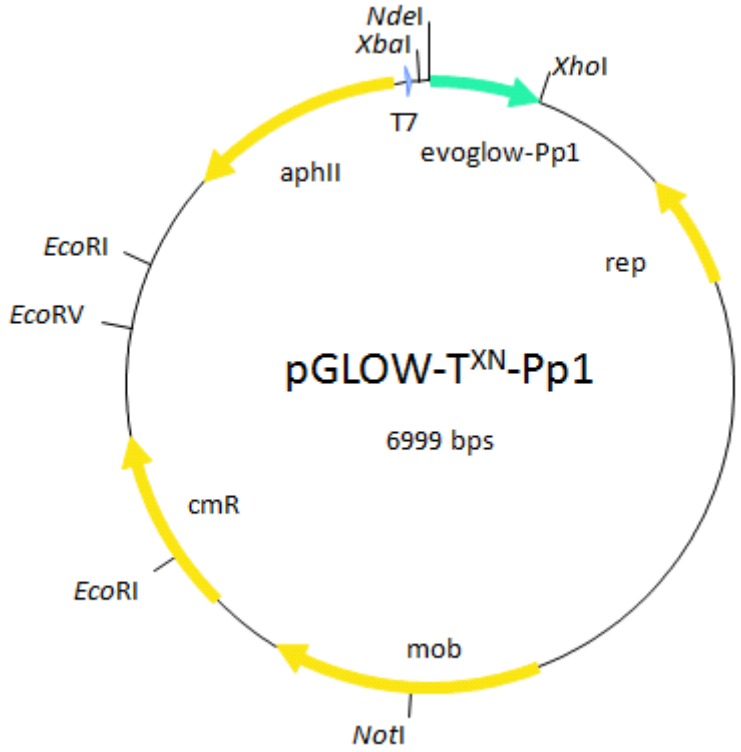
sequence landmarks:

marker	bp-positions	
rep	1356-	929
mob	3073-	4066
cm^R	4345-	5025
PaphII	6487-	5693
PT7	6871-	6886

XbaI *NdeI* *XhoI*

```
CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG GCT... evoglow-Bs2 ...TGC TCG AGC ACC
GGG AGA TCT TTA TTA AAA CAA ATT GAA ATT CTT CCT CTA TAT GTA TAC CGA...                ...ACG AGC TCG TGG
```

pGLOW-T^{XN}-Pp1



restriction analysis information:

enzyme	bp position	
*Eco*RI	4558	5667
*Eco*RV	5426	
*Nde*I	6956	
*Xba*I	6917	
*Xho*I	407	

sequence landmarks:

marker	bp-positions	
rep	1398-	971
mob	3115-	4108
cm^{R}	4387-	5067
PaphII	6529-	5735
PT7	6013-	6928

XbaI *NdeI* *XhoI*

```
CCC TCT AGA AAT AAT TTT GTT TAA CTT TAA GAA GGA GAT ATA CAT ATG GCT... evoglow-Pp1 ...CTC GAG CAC CAC
GGG AGA TCT TTA TTA AAA CAA ATT GAA ATT CTT CCT CTA TAT GTA TAC CGA...                ...GAG CTC GTG GTG
```

Licence Information

Limitation of use:

The plasmids distributed by company evocatal GmbH under the trade mark “evoglow®” are subject to intellectual property right applications. Company evocatal GmbH commits the plasmids and genes to the costumer solely for the purpose of own scientific, non-commercial investigation or own, non-commercial research and development purposes. Any commercial use requires the conclusion of a separate license contract. For commercial customers (i.e. companies or any for-profit-organisations) the permission of use for the above said research purposes of the evoglow genes and plasmids is limited to six (6) months, starting from the date of purchase. For detailed license information please contact evocatal GmbH, Germany. Furthermore, the modification of the evoglow-gene-sequences and of the plasmids is assertive prohibited. By the purchase of the plasmids without notice of defect within a term of 14 days beginning with the day of delivery, the customer agrees to the limitation of use above.

Exclusion of liability:

Company evocatal GmbH commits the plasmids distributed under the trade mark “evoglow®” to the customer solely for use in microorganism. The plasmids are not intended for the use in human medical or veterinarian medical diagnostic purposes, or in the animal or human body; any liability of the company evocatal GmbH is excluded. The committed plasmids are classified to security level S1. The national safety regulations for handling genetic engineered microorganism have to be considered and fulfilled when handling the plasmids. By the purchase of the plasmids without notice of defect within a term of 14 days beginning with the day of delivery, the customer agrees to the above exclusion of liability and indemnifies company evocatal GmbH from any liability for the use of the plasmids beyond the intended application.

Contact Information:

For any further information, questions or remarks please contact:

evocatal GmbH

Merowingerplatz 1a

40225 Düsseldorf

www.evocatal.com

info@evocatal.com