Plasmid RD-PGQZ5

Quantity: 50 µg supercoiled plasmid DNA, ethanol-precipitated and air dried.

Storage and Reconstitution: The dry pellet is shipped at room temperature. To reconstitute, spin the tube briefly and dissolve the contents in 50 µl of sterile, nuclease-free water to make a 1 µg/µl solution. Store at 4˚C.

Note: **A License from Molecular Devices Corporation is required prior to purchasing or using the plasmid.**

Description

Gαqz5 is a chimeric Gαq with the carboxy terminal amino acids changed from Gαq to Gαz residues (EYNLV to YIGLC). When expressed in transfected cells, the Gαqz5 protein allows many Gi/o-coupled receptors, which normally act through the cAMP pathway, to couple to Gq signal transduction and stimulate phospholipase C.

RD-PGQZ5 will be provided in two constructs with two different selectable markers (hygromycin, G418) to allow co-transfection with receptors.

pCEP-Gqz5-HA (hygromycin)

- 1.1-kb chimeric Gαqz5 cDNA in pCEP4 (vector size is 10.4-kb)
- Internal HA epitope in cDNA allows recognition by 12CA5 antibody (Boehringer Mannheim)
- Cytomegalovirus (CMV) enhancer/promoter for strong, constitutive expression of Gαqz5
- Hygromycin resistance gene for selection of stable transfected cells

- Epstein Barr Virus (EBV) origin of replication (oriP) and nuclear antigen (EBNA) for episomal replication in canine and primate cell lines
- Amp^r and ColE1 origin for selection and propagation in E. coli

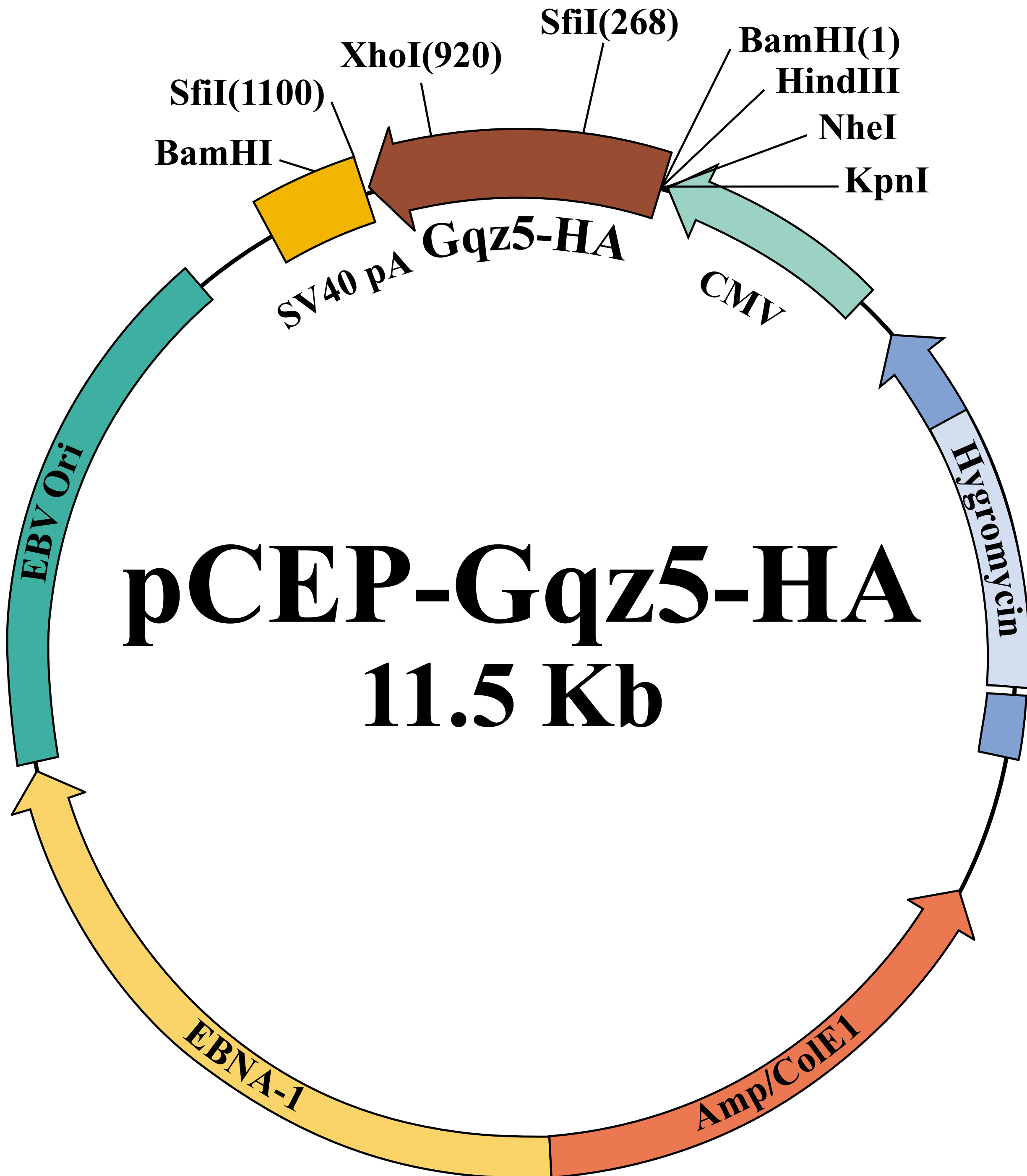
pLEC1-Gqz5-HA (G418)

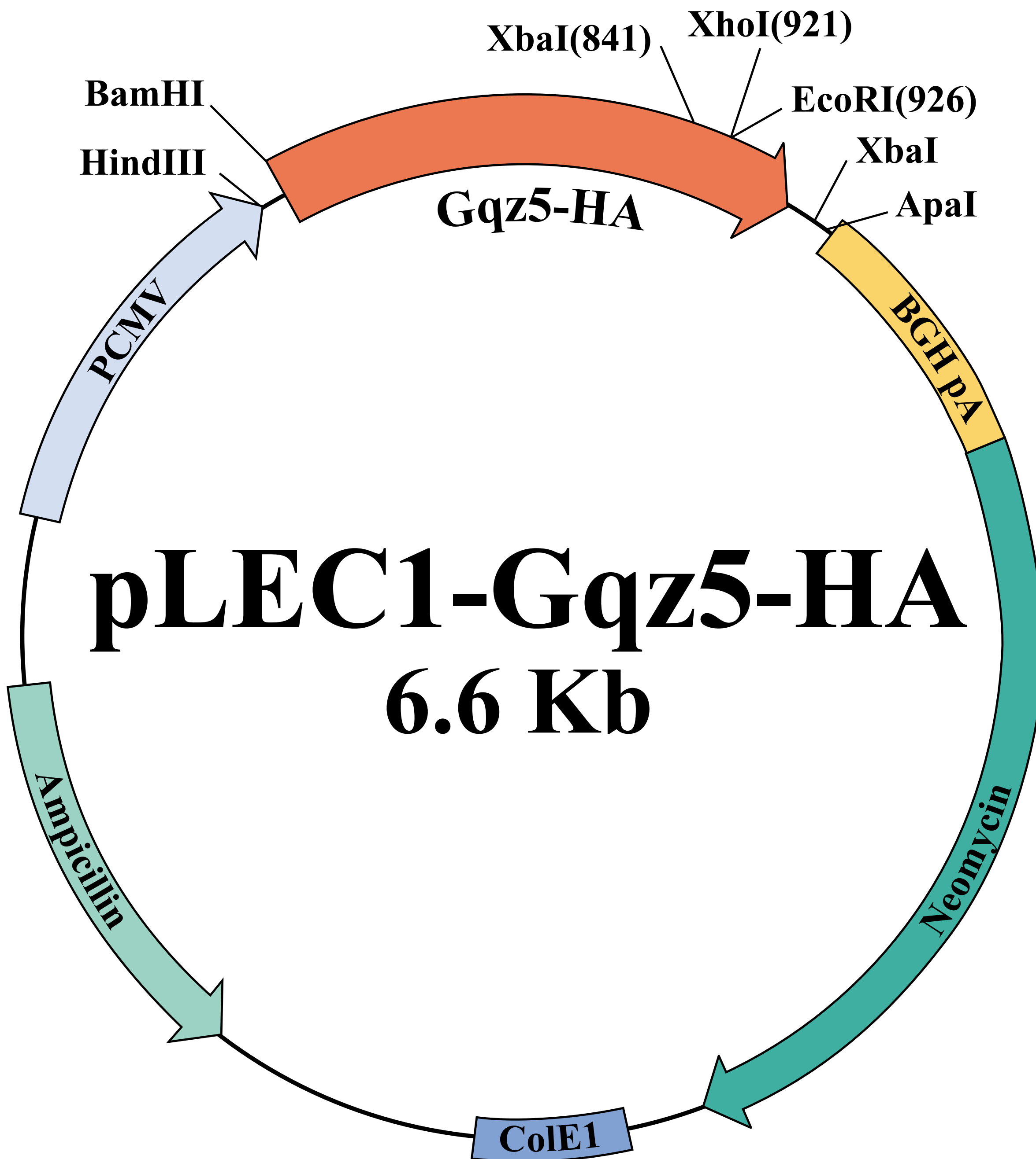
- 1.1-kb chimeric Gαqz5 cDNA in pLEC1 (vector size 5.5-kb)
- Internal HA epitope in cDNA allows recognition by 12CA5 antibody (Boehringer Mannheim)
- Cytomegalovirus (CMV) enhancer/promoter for strong, constitutive expression of Gαqz5
- Neomycin resistance gene for G418 selection of stable transfected cells
- T7 and SP6 promoters allowing *in vitro* synthesis of sense and antisense Gαqz5 RNA transcripts
- Amp^r and ColE1 origin for selection and propagation in E. coli

Quality Control

1. Identity and purity of the plasmid DNA is confirmed by restriction analysis on agarose gel.

2. Functional testing is carried out by transient transfection of the plasmid into CHO cells stably expressing the human nociceptin receptor (Gi/o-coupled) and measuring the stimulation of calcium mobilization by nociceptin in the FLIPR™ instrument (Molecular Devices Corporation, Sunnyvale, CA).


SfiI(268)
XhoI(920)
BamHI(1)
HindIII
SfiI(1100)
NheI
BamHI
KpnI
Gqz5-HA
SV40 pA
CMV
Hygromycin
EBV Ori
pCEP-Gqz5-HA
11.5 Kb
EBNA-1
Amp/ColE1


XbaI(841)
XhoI(921)
BamHI
EcoRI(926)
XbaI
HindIII
ApaI
Gqz5-HA
BGH pA
PCMV
pLEC1-Gqz5-HA
6.6 Kb
Ampicillin
Neomycin
ColE1