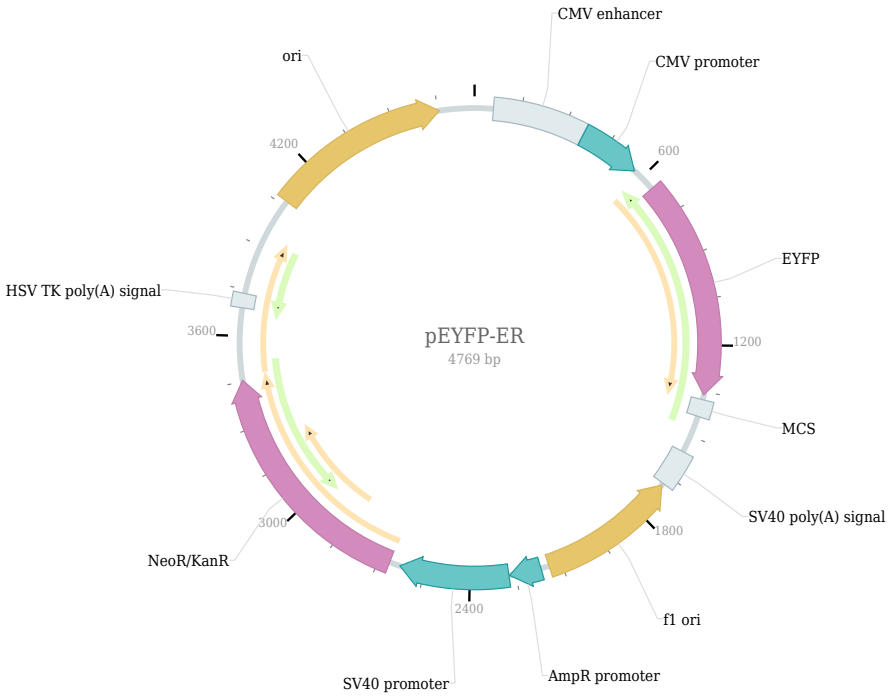
pEYFP-ER

Vector ID	V012636
Antibiotic Resistance	Kanamycin
Length	4769 bp
Vector Type	Fluorescent Protein Reporter Vectors

Product Information

Replication Origin	ori
Selection Marker	Neomycin
Promoter	CMV
Cloning Method	Enzyme digestion and ligation
Fusion Tag	EYFP
Growth Strain(s)	DH10B
Comment	pEYFP-ER encodes a fusion protein consisting of enhanced yellow fluorescent protein (EYFP); the endoplasmic reticulum (ER) targeting sequence of calreticulin (1), which is cloned at the 5' end; and the sequence encoding the ER retrieval sequence, KDEL (2, 3), which is cloned at the 3' end. The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (4, 5): Ser- 65 to Gly; Val-68 to Leu; Ser-72 to Ala; and Thr-203 to Tyr. The fluorescence excitation maximum of EYFP is 513 nm; the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 514 nm, the Emof EYFP is 84,500 cm–1M–1 and the fluorescence quantum yield is 0.61 (5), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that from EGFP.In addition to the chromophore mutations, pEYFP-ER contains more than 190 silent base changes that correspond to human codon-usage preferences (6). SV40 polyadenylation signals downstream of the EYFP-ER fusion direct proper processing of the 3' end of the mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neor) consisting of the SV40 early promoter, the neomycin/ kanamycin resistance gene of Tn5, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV-TK) gene allows stably transfected eukaryotic cells to be selected using G418 (7). A bacterial promoter upstream of this cassette drives expression of the gene encoding kanamycin resistance in E. coli. The pEYFP-ER backbone also provides a pUC origin of replication for propagation in E. coli and an f1 origin for single-stranded DNA production.pEYFP-ER is designed for fluorescent labeling of the ER in mammalian cells (8, 9). Fluorescence can be observed in living cells by microscopy or flow cytometry. pEYFP-ER can be introduced into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). Filter sets are available for dual-color detection of EYFP and ECFP using conventional epifluoresence microscopy (10). Please refer to the Living Colors User Manual for additional information.

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 μl for 5 μg plasmid, and 100 μl for 100 μg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.