pACT2 AD

Vector ID	V007324
Antibiotic Resistance	Ampicillin
Length	8117 bp
Vector Type	Yeast Two-Hybrid System
Host	Yeast

Product Information

Replication Origin	ori
Promoter	LEU2
Cloning Method	Enzyme digestion and ligation
Comment	pACT2 generates a fusion of the GAL4 AD (amino acids 768–881), an HA epitope tag, and a protein of interest (or protein encoded by a cDNA in a fusion library) cloned into the MCS in the correct orientation and reading frame. pACT2, which is derived from pACT (1), contains a unique EcoR I site in the MCS. The hybrid protein is expressed at high levels in yeast host cells from the constitutive ADH1 promoter (P); transcription is terminated at the ADH1 transcription termination signal (T). The protein is targeted to the yeast nucleus by the nuclear localization sequence from SV40 T-antigen which has been cloned into the 5’ end of the GAL4 AD sequence (2). pACT2 is a shuttle vector that replicates autonomously in both E. coli and S. cerevisiae and carries the bla gene, which confers ampicillin resistance in E. coli. pACT2 also contains the LEU2 nutritional gene that allows yeast auxotrophs to grow on limiting synthetic media. Transformants with AD/library plasmids can be selected by complementation by the LEU2 gene by using an E. coli strain that carries a leuB mutation (e.g., HB101).

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 ℃.