pGADT7 AD Vector Information

**Description**

pGADT7 AD is a yeast expression vector that is designed to express a protein of interest fused to a GAL4 activation domain (AD; amino acids 768–881). Transcription of the GAL4 AD fusion is driven by the constitutively active *ADH1* promoter (*P_{ADH1}*), and is terminated at the *ADH1* transcription termination signal (*T_{ADH1}*). The GAL4 AD fusion contains an N-terminal SV40 nuclear localization signal (SV40 NLS; 1) that targets the protein to the yeast nucleus, and a hemagglutinin epitope tag (HA Tag), located between the GAL4 AD and the protein of interest, that allows the protein to be easily detected with HA-tag antibodies.

The T7 promoter (*P_{T7}*), located just upstream of the HA tag sequence, allows *in vitro* transcription and translation of the HA-tagged protein of interest (without the GAL4 AD and the SV40 NLS). pGADT7 AD replicates autonomously in both *E. coli* and *S. cerevisiae* from the pUC and 2 μ ori, respectively. The vector also contains an ampicillin resistance gene (Amp') for selection in *E. coli* and a LEU2 nutritional marker for selection in yeast.

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Use

pGADT7 AD is the AD Cloning Vector provided in Clontech’s Matchmaker™ Gold Yeast One- and Two-Hybrid Screening Systems (Cat. Nos. 630491 and 630489, respectively). The vector allows the generation of GAL4 AD fusion proteins from either a gene of interest or a cDNA library. **Important:** Genes must be cloned into the MCS so that they are in-frame with the GAL4 AD and HA tag coding sequences. The vector can also be used as a negative “prey” control for the One-Hybrid System.

GAL4 AD/HA-tagged fusion proteins expressed by the vector can be detected with either our GAL4 AD Monoclonal Antibody (Cat. No. 630402) or our HA-Tag Polyclonal Antibody (Cat. No. 631207). Note: *In vitro* transcription/translation from the T7 promoter, located between the GAL4 AD and HA tag sequences, produces an HA-tagged protein that lacks the GAL4 AD. Such proteins can be detected by the HA-Tag Polyclonal Antibody, but not the GAL4 AD Monoclonal Antibody.

**Location of features**
- $P_{ADH1}$ (full-length *S. cerevisiae ADH1* promoter): 7–1478
- GAL4 AD (GAL4 activation domain with SV40 Nuclear Localization Signal [NLS])
  - SV40 NLS: 1500–1556
  - GAL4 (amino acids 768–881): 1560–1898
- $P_{T7}$ (T7 RNA polymerase promoter): 1904–1926
- HA Tag (hemagglutinin epitope tag): 1941–1967
- MCS (multiple cloning site): 1968–2040
- $T_{ADH1}$ (*S. cerevisiae ADH1*(Terminator)): 22879–2604
- LEU2 coding sequences: 2722–3813 (complementary)
- pUC ori (pUC replication origin): 4580–5417
- Amp' (ampicillin resistance gene): 5573–6433 (complementary)
- 2 μ ori (Yeast 2 μ replication origin): 6997–7987

**Location of primers**
- T7 Sequencing Primer: 1904–1924
- 3' AD Sequencing Primer: 2101–2082
- Matchmaker 5' AD LD-Insert Screening Amplimer (Cat. No. 630433): 1857–1888
- Matchmaker 3' AD LD-Insert Screening Amplimer (Cat. No. 630433): 2077–2045

**Propagation in *E. coli***
- Suitable host strains: DH5α, DH10 & other general purpose strains
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

**Propagation in *S. cerevisiae***
- Suitable host strains: Y1HGold, Y2HGold, AH109(*MATa*), Y187(*MATa*), Y190(*MATa*), SFY526(*MATa*), CG1945(*MATa*), HF7c(*MATa*)
- Selectable marker: LEU2
- *S. cerevisiae* origin: 2 μ

**Reference**

**Note:** The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

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