# pEF1α-tdTomato Vector

## Table of Contents



| Catalog No. | Amount | Lot Number |
|---|---|---|
| 631975 | 10 µg | Specified on product label. |

## Product Information

pEF1α- tdTomato is a mammalian expression vector that constitutively expresses the red fluorescent protein tdTomato, even after stable integration of the vector into the host cell genome. Stable, constitutive expression of tdTomato is driven by the human elongation factor 1 alpha (EF1α) promoter, which allows the protein to be expressed without the transgene silencing associated with CMV promoters. The vector, which lacks an MCS, is designed to be used for cell labeling or as a marker of transfection efficiency.

### Package Contents

- 1 tube of pEF1α-tdTomato Vector (20 µl/tube)

### Storage Conditions

- Store plasmid at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

### Shelf Life

- 1 year from date of receipt under proper storage conditions.

### Storage Buffer

- 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (pH 8.0)

### Concentration

- 500 ng/µl

### Shipping Conditions

- Dry ice (–70°C)

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(PA124333)

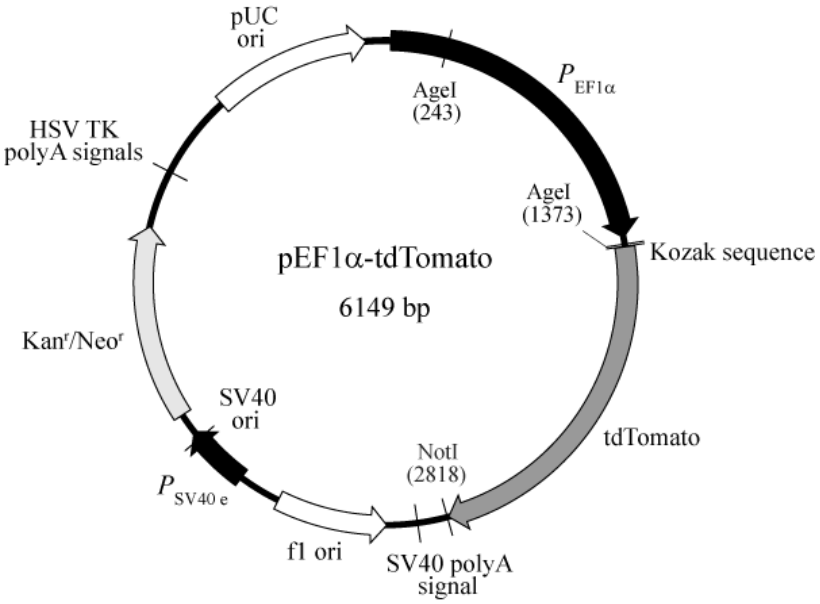


**Figure 1. pEF1α-tdTomato vector map.**

## Description

pEF1α-tdTomato is designed to constitutively express tdTomato in mammalian cells. tdTomato is a mutant fluorescent protein derived from the *Discosoma sp.* red fluorescent protein, DsRed (1, 2). The excitation and emission maxima of tdTomato are 554 nm and 581 nm, respectively.

The tdTomato coding sequence is positioned just downstream of the constitutively active EF1α promoter ($P_{EF1\alpha}$). As a result, mammalian cells transfected with this vector will constitutively express tdTomato, even after stable integration of the vector into the host cell genome (3). A Kozak consensus sequence located immediately upstream of the tdTomato coding sequence enhances translational efficiency of tdTomato in eukaryotic cells (4), and SV40 polyadenylation signals downstream of the tdTomato gene direct proper processing of the 3' end of the mRNA.

The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 large T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin-resistance cassette (Neo$^r$) allows stably transfected eukaryotic cells to be selected using G418 (5). This cassette consists of the SV40 early promoter ($P_{SV40\ e}$), the Tn5 neomycin/kanamycin resistance gene, and polyadenylation signals from the herpes simplex virus thymidine kinase (HSV TK) gene. A bacterial promoter upstream of the cassette drives expression of the kanamycin resistance gene in *E. coli*.

## Location of Features

- $P_{EF1\alpha}$ (human elongation factor 1 alpha promoter): 12–1346
- Kozak consensus sequence: 1378–1388
- tdTomato: 1385–2812
- SV40 polyA signal: 2968–3002
- f1 origin of replication: 3065–3520 (complementary)
- $P_{SV40\ e}$ (SV40 early promoter and enhancer sequences): 3694–3962
- SV40 origin of replication: 3861–3999
- Kan$^r$/Neo$^r$ (kanamycin/neomycin resistance gene): 4045–4839

- HSV TK polyA signals: 5075–5093
- pUC origin of replication: 5424–6067

## Additional Information

pEF1α-tdTomato is designed for use as a marker for cotransfection or for cell labeling. The red fluorescence of tdTomato can be detected by fluorescence microscopy, allowing direct visual imaging. In addition, flow cytometry can be used to enrich transfected cell populations. pEF1α-tdTomato can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (5).

### Propagation in *E. coli*

- Suitable host strains: DH5α, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid, such as the JM109 or XL1-Blue strains.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

### Excitation and Emission Maxima of tdTomato

- Excitation: 554 nm
- Emission: 581 nm

### References

1. Shaner, N. C. *et al*. (2004) *Nat. Biotechnol*. **22**(12):1567−72.
2. Bevis, B. J. & Glick, B. S. (2002) *Nat. Biotechnol*. **20**(1):83–87.
3. Wang, R. *et al.* (2008) *Stem Cells Dev.* **17**(2):279−289.
4. Kozak, M. (1987) *Nucleic Acids Res*. **15**(20):8125–8148.
5. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*. Ed. D. M. Glover (IRL Press, Oxford, U.K.) pp. 143–190.

## Quality Control Data

### Plasmid Identity & Purity

- Digestion with the indicated restriction enzymes produced fragments of the indicated sizes on a 0.8% agarose/EtBr gel:

| Enzyme(s) | Fragment(s) |
|---|---|
| NotI | 6.1 kb |
| AgeI | 1.1 & 5.0 kb |

- Vector identity was confirmed by sequencing.
- $A_{260}/A_{280}$: 1.8–2.0

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## pEF1alpha-tdTomato Vector

### CATALOG NO.

**631975**

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