

## Certificate of Analysis

### pF25A ICE T7 Flexi® Vector:

**Part No.**                      **Size (units)**  
L106A                              20µg

**Description:** The pF25A ICE T7 Flexi® Vector<sup>(a-1)</sup> is designed to be used with the TnT® T7 Insect Cell Extract Protein Expression System (Cat.# L1101, L1102) for maximal protein expression in this eukaryotic cell-free protein expression system.

The pF25A ICE T7 Flexi® Vector contains the following features:

- A T7 RNA polymerase promoter for cell-free protein expression in the TnT® T7 Insect Cell Extract Protein Expression System.
- 5' and 3' untranslated region (UTR) sequences from a baculovirus polyhedrin gene (1,2) and a synthetic poly(A) tract for enhanced translation in the TnT® T7 Insect Cell Extract Protein Expression System.
- The lethal barnase gene for positive selection of the insert. **Note:** The pF25A ICE T7 Flexi® Vector can only be propagated in *E. coli* once the barnase gene has been replaced with the protein-coding sequence of interest.
- An ampicillin-resistance gene for selection of the plasmid.
- Unique SgfI and PmeI sites, which allow easy insertion of the sequence of interest and transfer to and from other Flexi® Vectors with different expression options.

**Concentration:** 100ng/µl.

**GenBank® Accession Number:** EU754721.

**Storage Buffer:** The pF25A ICE T7 Flexi® Vector is supplied in 10mM Tris-HCl (pH 8.0), 1mM EDTA.

**Storage Conditions:** See the Product Information Label for storage recommendations. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability. See the label for the expiration date.

#### Usage Notes:

1. This vector was designed to be used with the Flexi® Vector System, a directional cloning method to shuttle protein-coding sequences between compatible vectors. The protein coding region can be cloned into the pF25A ICE T7 Flexi® Vector using the Flexi® System, Entry/Transfer (Cat.# C8640). For more information, see the *Flexi® Vector Systems Technical Manual #TM254*, available online at: [www.promega.com/protocols/](http://www.promega.com/protocols/)
2. Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

## Quality Control Assays

### Contaminant Assays

**Contaminating Nuclease Assay:** RNA, single-stranded DNA and chromosomal DNA are not evident in specified quantities of this vector as determined by agarose gel electrophoresis.

**Nuclease Assay:** Following incubation of 1µg of the vector in Restriction Enzyme Buffer at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

**Physical Purity:**  $A_{260}/A_{280} \geq 1.80$ ,  $A_{260}/A_{250} \geq 1.05$ .

### Functional Assays

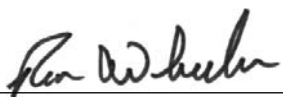
**Identity Assay:** The vector has been sequenced completely and has 100% identity with the published sequence available at: [www.promega.com/vectors/](http://www.promega.com/vectors/)

**Restriction Digestion:** The functional purity of the vector is verified by successful digestion with SgfI at the optimal temperature for one hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

## References

1. Ezure, T. *et al.* (2006) Cell-free protein synthesis system prepared from insect cells by freeze-thawing. *Biotechnol. Prog.* **22**, 1570–77.
2. Suzuki, T. *et al.* (2006) Performance of expression vector, pTD1, in insect cell-free translation system. *J. Biosci. Bioeng.* **102**, 69–71.

Signed by:



R. Wheeler, Quality Assurance

Part# 9PIL106

Revised 4/18



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**Promega**

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## pF25A ICE T7 Flexi® Vector Features and Circle Map

The following features are present in the vector based on nucleotide sequence.

T7 RNA polymerase promoter (-17 to +2)	21-39
5' polyhedrin UTR	40-85
Sgfl site	86-93
Barnase coding region	117-452
PmeI site	454-461
3' polyhedrin UTR	610-983
Synthetic poly(A) region	988-1017
T7 terminator	1018-1065
β-lactamase (Amp <sup>r</sup> ) coding region	1446-2306
ColE1-derived plasmid origin of replication	2461-2497
cer site (site for <i>E. coli</i> XerCD recombinase)	3168-3453

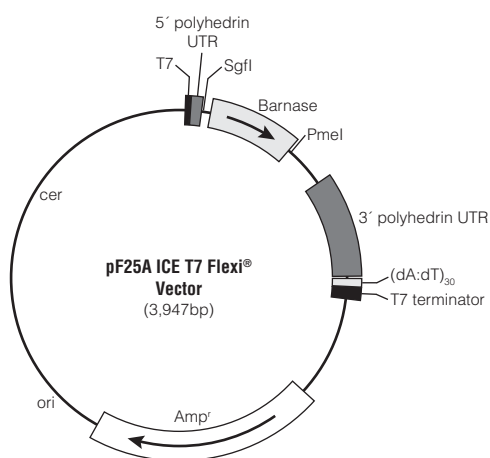


Figure 1. pF25A ICE T7 Flexi® Vector circle map and sequence reference points.

<sup>(a)</sup>Patent Pending.

<sup>(b)</sup>For research use only. Persons wishing to use this product or its derivatives in other fields of use, including without limitation, commercial sale, diagnostics or therapeutics, should contact Promega Corporation for licensing information.

<sup>(c)</sup>Ezure, T., Suzuki, T., Higashide, S., Shintani, E., Endo, K., Kobayashi, S., Shikata, M., Ito, M., Tanimizu, K., Nishimura, O. (2006) Cell-free protein synthesis system prepared from insect cells by freeze-thawing. *Biotechnol. Prog.* **22**, 1570-7.

<sup>(d)</sup>Use of this vector for applications outside of the TnT® T7 Insect Cell Extract Protein Expression System may require a license from Shimadzu Corporation. For more information contact t-direct@shimadzu-biotech.jp

<sup>(e)</sup>European Pat. No. 1685247 and other patents pending.

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