

## Certificate of Analysis

### pF25K ICE T7 Flexi® Vector:

**Part No.** L108A  
**Size (units)** 20µg

**Description:** The pF25K ICE T7 Flexi® Vector<sup>(a-e)</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 pF25K 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 pF25K 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.
- A kanamycin-resistance gene for selection of the plasmid.
- Unique SgfI and Pml 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:** EU754722.

**Storage Buffer:** The pF25K 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 pF25K 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.

Part# 9PIL108

Revised 4/18



AF9PIL108 0418L108



**Promega**

#### Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	<a href="http://www.promega.com">www.promega.com</a>

## 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

#### PRODUCT USE LIMITATIONS, WARRANTY, DISCLAIMER

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

© 2008–2018 Promega Corporation. All Rights Reserved.

Flexi and TnT are registered trademarks of Promega Corporation.

GenBank is a registered trademark of US Dept of Health and Human Services.

All specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

Part# 9PIL108  
Printed in USA. Revised 4/18.

## pF25K 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
Kanamycin resistance (Kan <sup>r</sup> ) coding region	1493-2287
ColE1-derived plasmid origin of replication	2456-2492
cer site (site for <i>E. coli</i> XerCD recombinase)	3163-3448

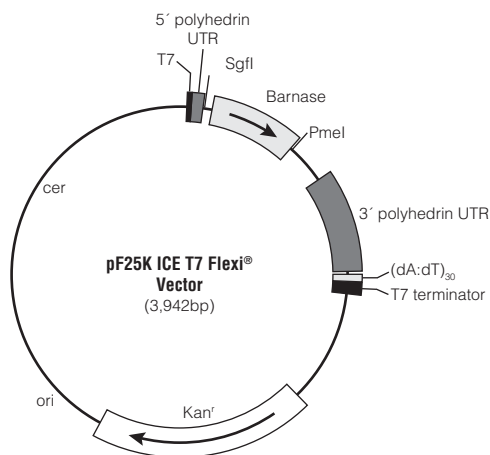


Figure 1. pF25K ICE T7 Flexi® Vector circle map and sequence reference points.

<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.

<sup>(f)</sup>For Research Use Only. Not for Use in Diagnostic Procedures.