

## Certificate of Analysis

### pF3K WG (BYDV) Flexi® Vector:

**Part No.** L568A  
**Size (units)** 20µg

**Description:** The pF3K WG (BYDV) Flexi® Vector<sup>(e-d)</sup> is designed for use with the Flexi® System, Transfer (Cat.# C8820), and the Flexi® System, Entry/Transfer (Cat.# C8640). The vector contains translation enhancer (TE) sequences from the barley yellow dwarf virus (BYDV), an RNA plant virus, that facilitate in vitro expression of protein in wheat germ extract. T7 and SP6 RNA polymerase promoters allow production of RNA for subsequent translation applications. Linear DNA templates for in vitro RNA transcription can be produced by cutting at one of the restriction enzyme sites located downstream of the 3' BYDV TE region. The vector also contains the lethal barnase gene for positive selection of the insert, a kanamycin-resistance gene for selection of the plasmid in *E. coli* and unique SgfI and PmeI sites, which allow easy insertion or transfer of the sequence of interest. Inserts containing a protein-coding region can easily be transferred from the pF3K WG (BYDV) Flexi® Vector to other Flexi® Vectors with different expression options (Table 1). For more information, see the *Flexi® Vector Systems Technical Manual* #TM254 or the *Wheat Germ Extract Plus Technical Manual* #TM066.

**Table 1. Vectors Available for Use With the Flexi® Vector Systems.**

Cat.#	Flexi® Vector	Utility	Expression	Drug Selection
C8441	pF1A T7 Flexi® Vector	Protein expression	<i>E. coli</i> and in vitro (T7 promoter)	Ampicillin
C8451	pF1K T7 Flexi® Vector			Kanamycin
C8461	pFN2A (GST) Flexi® Vector	Protein expression	<i>E. coli</i> and in vitro (T7 promoter)	Ampicillin
C8471	pFN2K (GST) Flexi® Vector	and purification		Kanamycin
L5671	pF3A WG (BYDV) Flexi® Vector	Protein expression	Wheat germ extract in vitro (T7, SP6 promoter)	Ampicillin
L5681	pF3K WG (BYDV) Flexi® Vector	Protein expression	Wheat germ extract in vitro (T7, SP6 promoter)	Kanamycin
C8481	pF4A CMV Flexi® Vector	Protein expression	Mammalian (CMV promoter)	Ampicillin
C8491	pF4K CMV Flexi® Vector		and in vitro (T7 promoter)	Kanamycin

### Usage Information

**Concentration:** 100ng/µl.

**GenBank® Accession Number:** AY949044.

**Storage Buffer:** The pF3K WG (BYDV) Flexi® Vector is supplied in 10mM Tris-HCl (pH 8.0), 1mM EDTA.

**Storage Conditions:** Store the vector at -20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

**Usage Notes:** Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

### Quality Control Assays

**Nuclease Assay:** Following incubation of 1µg of pF3K WG (BYDV) Flexi® Vector in Restriction Enzyme Buffer B at 37°C for 16 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

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

**Restriction Digestion:** The presence of unique restriction sites for PmeI and SgfI is confirmed by showing that the vector yields the expected fragment sizes after digesting 1µg of vector for 2 hours with 10 units of PmeI, SgfI and BgIII.

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

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

<sup>(c)</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>(d)</sup>Licensed under U.S. Pat. No. 5,910,628.

Signed by:

R. Wheeler, Quality Assurance

Part# 9PIL568

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## pF3K WG (BYDV) Flexi® Vector Features and Circle Map

pF3K WG (BYDV) Flexi® Vector sequence reference points:

SP6 RNA polymerase promoter (-17 to +3)	23-42
T7 RNA polymerase promoter (-17 to +1)*	46-63
BYDV 5' UTR	65-201
SgfI site	202-209
barnase coding region	233-568
PmeI site	570-577
BYDV 3' TE	596-702
T7 terminator	807-854
kanamycin resistance gene	1235-2029
origin of replication	2198-2234
cer site (site for <i>E. coli</i> XerCD recombinase)	2905-3190
rrnB transcription terminator	3241-3642

**\*Note:** The consensus T7 RNA polymerase promoter extends only to +1 and does not match many standard T7 promoter primers available for sequencing applications. However, the SP6 Promoter Primer (Cat.# Q5011) can be used to sequence inserts cloned into the pF3K WG (BYDV) Flexi® Vector.

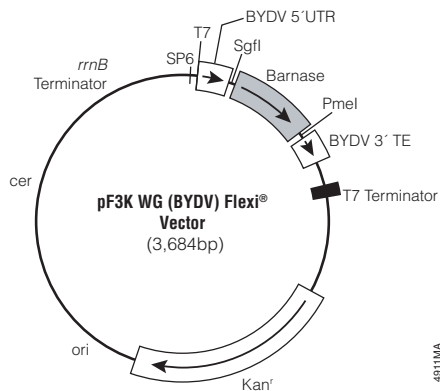


Figure 1. pF3K WG (BYDV) Flexi® Vector circle map and sequence reference points.