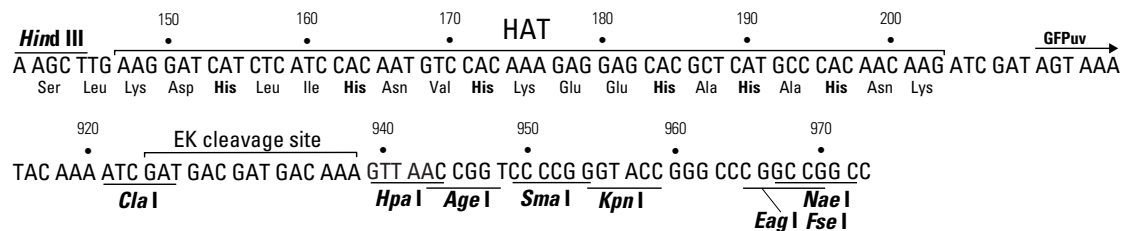
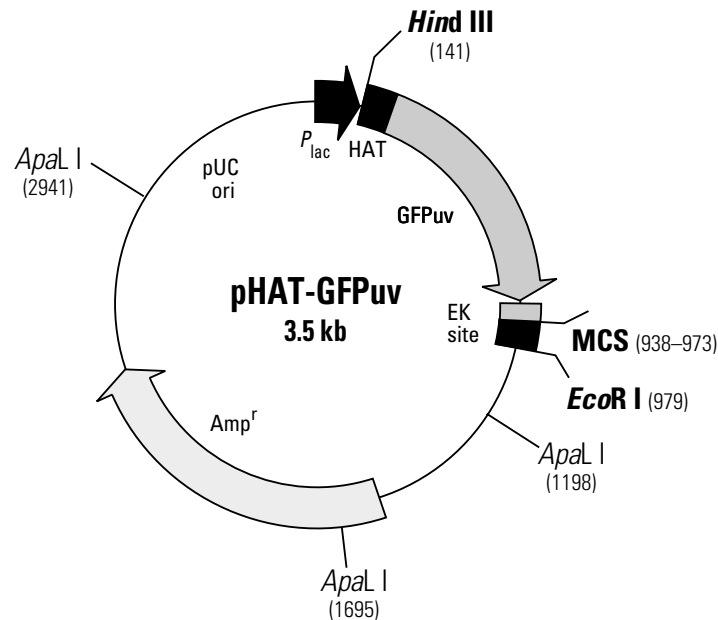


## pHAT-GFPuv<sup>+</sup> Vector Information

GenBank Accession #: Submission in progress.

PT3312-5

Catalog #8920-1



**Restriction Map and MCS of pHAT-GFPuv.** Unique restriction sites are in bold. The EK cleavage site is located between the HAT-GFPuv fusion and the MCS for quick isolation of your native protein.

### Description:

The pHAT-GFPuv Vector encodes a novel Histidine Affinity Tag (HAT<sup>TM</sup>) epitope, which enables purification of expressed proteins at neutral pH. pHAT-GFPuv encodes a variant of the *Aequorea victoria* green fluorescent protein (GFP) that has been optimized for brighter fluorescence when excited by standard UV light (1). Therefore, HAT-GFPuv fusion proteins can be visually monitored during the protein purification process.

The HAT epitope is a naturally occurring, 19-amino-acid sequence from the chicken lactate dehydrogenase protein. This sequence of nonadjacent histidine residues has lower overall charge than tags with consecutive His residues, such as the 6xHis tag. As a result, HAT-GFPuv fusion proteins exhibit solubility that more closely resemble those of wild-type proteins while still possessing strong affinity for immobilized metal ions. The unique binding characteristics of the HAT sequence allow both imidazole- and pH-gradient purification of proteins under native conditions at neutral pH (7.0), as well as under denaturing conditions.

The HAT sequence and an enterokinase (EK) cleavage site have been incorporated into the pUC19 backbone. The EK site allows for optional removal of the HAT-GFPuv sequence from the purified protein by treatment with enterokinase. Restriction sites allow excision of the HAT-GFPuv sequence, with or without the EK site, for cloning into other vectors.

**Use:**

Proteins expressed from the pHAT-GFPuv Vectors can be purified with our TALON® IMAC Resin using batch or gravity-flow protocols, or with our TALON® Superflow™ Resin using medium-pressure FPLC. TALON has a remarkably high affinity for His-tagged proteins and very low affinity for other proteins (2–3). The unique binding properties of the cobalt metal in TALON and of the HAT sequence combine to deliver purification without the need for an additional wash step and under pH conditions that preserve protein integrity.

**Location of Features:**

- $P_{lac}$  promoter: 1–84
- Histidine affinity tag (HAT): 147–203
- GFPuv reporter gene: 210–920
- Enterokinase cleavage site: 924–938
- MCS: 938–973
- Ampicillin resistance gene:  
    β-lactamase coding sequences:  
    start codon (ATG): 1579–1581; stop codon: 2437–2439

**Propagation in *E. coli*:**

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC

**References:**

1. Cramer, A., *et al.* (1996) *Nature Biotechnol.* **14**:315–319.
2. HAT Vectors & Sequencing Primers (January 1999) *CLONTECHniques XIV*(1):28–29.
3. HAT Protein Expression & Purification System (July 1998) *CLONTECHniques XIII*(3):27–28.

†Patent pending

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The attached sequence file has been 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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