



Map and MCS of pHAT10/11/12. (Unique restriction sites are in bold). The sequence of pHAT10 is shown. The asterisk indicates the location of additional bases in pHAT11 (G) and pHAT12 (GG) that alter the reading frame of the MCS.

Description

The pHAT Vectors are the core of the HAT (Histidine Affinity Tag) Protein Expression and Purification System. These vectors encode a novel polyhistidine epitope tag that enables purification of expressed proteins at neutral pH. The pHAT vectors allow protein purification under both native and denaturing conditions.

The HAT epitope is a naturally occurring, 19 amino acid sequence from the chicken lactate dehydrogenase protein. This sequence of nonadjacent histidine residues has a lower overall charge than tags with consecutive histidine residues, such as the 6 x histidine tag. As a result, HAT fusion proteins exhibit solubilities that more closely resemble those of wild-type proteins while still possessing a 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 sequence from the purified protein by treatment with enterokinase. Restriction sites allow excision of the HAT sequence, with or without the EK site, for cloning into other vectors.

Note: The attached sequence is provided for pHAT10. The sequences for pHAT11 and pHAT12 differ by additional bases that alter the reading frame in the MCS as indicated in the figure above.

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Use

Proteins expressed from the pHAT 10/11/12 Vectors can be purified with TALON® Resin, using batch or gravity flow protocols, or with Talon® Superflow Resin, using medium-pressure FPLC. Talon® resins have a remarkably high affinity for histidine-tagged proteins and a very low affinity for other proteins. The unique binding properties of the cobalt metal ion in Talon® resins and those of the HAT sequence combine to deliver purification under pH conditions that preserve protein integrity, without the need for an additional wash solution.

Location of Features

- P_{lac} promoter: 1–84
- Histidine affinity tag (HAT): 147–203
- Enterokinase cleavage site: 207–221
- MCS: 222–265
- Ampicillin resistance gene: 792–1722
 β -lactamase coding sequences: 862–1722

Propagation in *E. coli*

- Suitable host strains: DH5 α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 μ g/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC

Note: 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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