### pET28-MKH8SUMO Vector (GenBank accession N/A)

<table>
<thead>
<tr>
<th>Source</th>
<th>Constructed by Yufeng Tong</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
<td>Structural Genomics Consortium, Toronto</td>
</tr>
</tbody>
</table>

**Description**
The pET28-MKH8SUMO vector was derived from expression plasmid pET28a-LIC (SGC). It is used for T7 promoter driven expression of recombinant proteins with the addition of an N-terminal fusion tag containing 8X His followed by a thrombin cleavage site, a SUMO, and a TEV cleavage site. Two lysines were encoded after the Met start site. Two stop codons are included in the vector at the downstream cloning site.

**Antibiotic resistance**
Kanamycin, 50 μg/ml

**Promoter**
T7 - lacI

**Cloning Methods**
Insertion of DNA sequence into the cloning/expression region is performed using ClonTech In-Fusion™ enzyme mediated directional recombination between complementary 15 nucleotide DNA sequences at the ends of the insert (PCR product) and BseRI/Bsal linearized vector. Insertion of target sequence involves replacement of a SacB gene stuffer sequence, which provides for negative selection of the original plasmid on 5% sucrose.

**Initiation Codon**
ATG site in vector at bp 5070, NcoI site was destroyed

**N-terminal fusion sequence**
mkkhhhhhhhshsglypgrsmsdsevnqeakpevkpevkpevskdggseifffkkktpprlmeafakrgkemdsbrflydgqrqadqpeddmedn
diicahreqigggenlyfg

**Termination codons**
TGATGA included in 3’ PCR primer and vector cloning site. No amino acid residues added at cloning junction

**Additional features**
The 15-bp primer extensions are compatible with those for pET28-MHL, such that the PCR inserts prepared for pET28-MHL can be directly used for cloning into pET28-MKH8SUMO.

**Preferred Hosts**

<table>
<thead>
<tr>
<th>5’ primer for amplification of insert</th>
<th>5’ TTG TAT TTC CAG GGC --- 3’</th>
</tr>
</thead>
<tbody>
<tr>
<td>3’ primer for amplification of insert</td>
<td>5’ CAA GCT TCG TCA TCA --- 3’</td>
</tr>
<tr>
<td>5’ sequencing primer T7-Fwd</td>
<td>5’ AATAATACGACTCACTATAGGG 3’</td>
</tr>
<tr>
<td>3’ sequencing primer T7-Rev</td>
<td>5’ ATGCTAGTTATTGGCTAGCGG 3’</td>
</tr>
</tbody>
</table>
pET28-MKH8SUMO vector map

- T7 promoter: 4980-5002
- N-terminal tag: 5070-5447
- Upstream Bsal site: 5454-5449
- Downstream BseRI site: 7140-7154
- T7 terminator: 7254-7300
- f1 origin: 12-467
- KmR coding sequence: 562-1374
- ColE1 replicon: 1485-2099
- lacI coding sequence: 3517-4596
- sacB coding sequence: 5969-7387
pET28-MKH8SUMO cloning/expression region

**Upstream cloning site:**

```
CTAGAGGATCCGATCTCTGATCCTCGCCGGCGAATTAATACGACTCACTATAGGATTTGGTGAAGCGGATTAACAAATTC

5'305 5'320 5'330 5'340 5'350 5'360 5'370
```

**Downstream cloning site:**

```
AGACCACTGCTTTTAAAGAGGACATGGGATGAGGAACTGAAGATCCTGTCCTCCAGCTGGGATATCCATGGGATTAACAAATTC

5'305 5'320 5'330 5'340 5'350 5'360 5'370
```