

pSNAP-tag[®] (T7)-2 Vector



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N9181S 001150118011

N9181S

20 µg

Lot: 0011501

Store at: -20°C

Exp: 1/18

Introduction

pSNAP-tag (T7)-2 Vector is an *Escherichia coli* expression plasmid encoding the SNAP-tag protein. Expression is under control of the IPTG inducible T7 promoter. The pSNAP-tag(T7)-2 Vector can also be used as a control plasmid expressing the SNAP-tag protein (20 kDa). The target gene should be cloned as a fusion to the N- or C-terminus of the SNAP-tag.

The SNAP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest.

The SNAP-tag is a protein based on human O⁶-alkylguanine-DNA-alkyltransferase (hAGT)(1,2,3). SNAP-tag substrates are derivatives of benzyl purines and benzyl pyrimidines. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag.

There are two steps to using this system: subcloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice. Cloning and expression of SNAP-tag fusion proteins are described in this document. The labeling of the fusion proteins with SNAP-tag substrates is described in the instructions supplied with SNAP-tag substrates.

Materials Required but not Supplied:

General molecular biology supplies and equipment
IPTG inducible *E. coli* strains:

T7 Express Competent *E. coli* (NEB #C2566)

T7 Express ^h Competent *E. coli* (NEB #C3016)

T7 Express *lysY* Competent *E. coli* (NEB #C3010)

T7 Express *lysY/h* Competent *E. coli* (NEB #C3013)

Storage

pSNAP-tag(T7)-2 Vector is supplied in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) at a concentration of 0.5 µg/µl. Plasmid solutions can be stored at 4°C for up to one week. For long-term storage -20°C is recommended.

Detailed Description

A plasmid map and the sequence of the cloning region can be found at the end of these instructions. The complete plasmid sequence can be downloaded at www.neb.com. This plasmid encodes the gene *SNAP26b* which is a mutant form of the human gene for O⁶-alkylguanine-DNA-alkyltransferase (hAGT) (Compared to wild type hAGT, the SNAP-tag protein contains the mutations C26A, K125A, A127T, R128A, G131K, G132T, M134L, R135S, C150S, N157G, S159E, and is truncated after G182). The codon usage of the gene is optimized for expression in *E. coli*. In the plasmid sequence, the *SNAP26b* gene is encoded from bp 5767 to 6311. It is not flanked by additional tags for purification. The plasmid contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322 (at a moderate to low copy number of 20 copies/cell). It encodes an ampicillin resistance gene.

This plasmid is intended as an expression vector for SNAP-tag gene fusions in appropriate *E. coli* strains. The strain needs to provide T7 polymerase in order to achieve expression from the T7 promoter upstream of the gene (4). Expression of the SNAP-tag in pSNAP-tag(T7)-2 has been tested in the following *E. coli* strains supplied by NEB: T7 Express Competent *E. coli* (NEB #C2566), T7 Express ^h Competent *E. coli* (NEB #C3016), T7 Express *lysY* Competent *E. coli* (NEB #C3010) and T7 Express *lysY/h* Competent *E. coli* (NEB #C3013). We recommend using T7 Express strains to conduct initial tests of the expression of the fusion gene.

Cloning of SNAP-tag Fusions in pSNAP-tag(T7)-2

Cloning by PCR

The protein of interest can be expressed with the SNAP-tag (20 kDa) as either an N- or a C-terminal fusion.

For fusion to the C-terminus of the SNAP-tag, subclone your gene of interest into the 3' MCS of the pSNAP-tag(T7)-2 Vector using the available restriction sites SbfI, PstI, BamHI, XmaI, SmaI, XhoI and PacI which are located upstream of the stop codon, or NotI downstream of the stop codon in 3' MCS. Include a stop codon where necessary. To subclone the gene of interest into pSNAP-tag(T7)-2 fused to the N-terminus of the SNAP-tag use the

available restriction sites NdeI, NheI, NcoI, EcoRV, HindIII, AgeI and EcoRI which are located upstream of the SNAP-tag.

Primer Design and Cloning Hints:

- Design your PCR primers to include a sufficient overlap (15–20 bp) with the sequence of the gene you want to amplify.
- Care should be taken to design the cloning so that the fusion partners in the resulting construct are in frame.
- Adapt the flanking regions to the cloning destination in pSNAP-tag(T7)-2. If you add an upstream SbfI and a downstream XbaI site in the primers, cloning downstream of *SNAP26b* in pSNAP-tag(T7)-2 should be straightforward.
- For fusions to the C-terminus of the SNAP-tag you may also want to include a stop codon at the C-terminus of the fusion (in front of the downstream cloning site) in order to terminate translation at this position.
- After subcloning the gene of interest into pSNAP-tag(T7)-2 as a fusion with the *SNAP26b* gene, the resulting plasmid can be used for expression of the SNAP-tag fusion proteins in a suitable *E. coli* host strain.
- In general, any linker peptide between the proteins should be kept short to avoid degradation by proteases. If required, specific protease cleavage sites can be introduced into the linker peptide.
- Perform the PCR reaction and subsequent cloning steps according to established protocols for molecular biology.

Direct Cloning

Direct cloning can also be used to make fusions with the SNAP-tag. This is possible if the gene of interest is flanked by sites compatible with the polylinker in pSNAP-tag(T7)-2.

The sequence of the *SNAP26b* gene is flanked by a number of restriction sites which can be found on the plasmid map.

Expression of SNAP-tag Fusions

This plasmid can serve as an expression vector for SNAP-tag fusions or the SNAP-tag alone (20 kDa) in appropriate *E. coli* strains. pSNAP-tag(T7)-2 can be used for expression in all NEB T7 Express strains: T7 Express Competent *E. coli* (NEB #C2566), T7 Express ^h Competent *E. coli* (NEB #C3016), T7 Express *lysY* Competent *E. coli* (NEB #C3010) and T7 Express *lysY/h* Competent *E. coli* (NEB #C3013).

The following instructions have been developed from our experience with the expression of various SNAP-tag fusion proteins and the SNAP-tag. However, if your particular fusion protein requires specific conditions that are not compatible with the ones mentioned below, it is useful to try test expressions and determine whether the SNAP-tag activity is retained under these conditions.

1. In order to express the SNAP-tag or a fusion protein from the provided vector, transform the plasmid into an *E. coli* expression strain that provides inducible T7 polymerase.
2. Pick a colony of the transformed cells the next day and grow in LB containing ampicillin (100 mg/L at 37°C) until the OD₆₀₀ reaches approximately 0.5.
3. Induce expression of the T7 polymerase by adding IPTG to a final concentration of 0.4 mM. This will lead to expression of genes under control of the T7 promoter.
4. Shake the culture at 25 to 30°C a further 3 to 8 hours or 15°C for 16 hours. The yield of soluble protein is usually increased at a reduced temperature (or in conjunction with lower IPTG concentration, e.g. 0.05–0.1 mM) for a number of SNAP-tag fusion proteins.
5. Harvest the bacteria by centrifuging the culture for 15 min at 5000 x g. The bacterial pellets may be stored frozen at -20°C prior to lysis.
6. The cells can be lysed and the fusion protein extracted by standard methods.
7. The crude lysate can be used directly to perform the SNAP-tag reaction in the presence of 10 µM substrate.

Purification

SNAP-tag fusion proteins can be purified before labeling, but the labeling reaction also works in non-purified protein solutions (including cell lysates). The SNAP-tag, as provided in the vector, does not contain an affinity tag, so it should be purified by standard separation methods of protein chemistry (e.g. ion exchange chromatography, gel filtration). If you have incorporated an affinity tag into your fusion protein, use protocols adapted to that affinity tag.

We recommend the routine addition of 1 mM DTT or another reducing reagent (such as β-mercaptoethanol at 1–5 mM) to solutions used for (see other side)

the purification and storage of SNAP-tag fusion proteins. SNAP-tag stability and reactivity *in vitro* is improved by the presence of a reducing reagent.

The presence of chelating reagents such as EDTA should be avoided in solutions used for the expression, purification, and reaction of the SNAP-tag, as the protein contains a structural Zn²⁺ ion.

Storage and Handling of Unlabeled SNAP-tag Fusion Proteins

Correct storage and handling of unlabeled SNAP-tag fusion proteins is essential to maintain reactivity of the SNAP-tag prior to labeling.

Add 1 mM DTT or another reducing reagent to buffers used for the storage of unlabeled SNAP-tag fusion proteins. Unlabeled protein samples should be stored at -20°C, or at -80°C for long-term storage. Handling at temperatures above 0°C should be minimized by thawing the unlabeled protein samples shortly before use, and keeping them on ice until just before the labeling reaction.

If a particular fusion protein requires buffers without DTT or another reducing agent, minimize handling the protein above 4°C before the labeling reaction itself.

The SNAP-tag itself is tolerant of a wide range of buffers. The requirements of your fusion partner should dictate the buffer selected. From our experience, the following storage buffer composition gives good performance, especially when freezing protein material: pH between 7.0 and 8.0, monovalent salts (e.g. sodium chloride) between 50 mM and 250 mM, and at least 1 mM DTT. Non-ionic detergents can be added if required, but ionic detergents should be avoided because they reduce the activity of the SNAP-tag.

Labeling of SNAP-tag Fusion Proteins

The labeling of the fusion protein in solution with SNAP-tag substrates can be performed at 16–37°C for 30 minutes in the presence of 10 µM substrate, 5 µM SNAP-tag, 1 mM DTT and 1X PBS or 1X SNAP-tag reaction buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1% Tween-20). It is also possible to label a SNAP-tag fusion protein at 4–16°C; however, an optimization of various parameters (e.g. labeling time, substrate and protein concentrations) should be carried out.

Troubleshooting

Cloning of SNAP-tag

If subcloning of the SNAP-tag with your gene of interest does not work, reconfirm all the cloning steps (primer design, choice of restriction site, etc.) If all steps are confirmed as being correct, then try the cloning using different restriction sites. Be sure to include a positive and negative control for the ligation reaction.

Alternatively, subclone the SNAP-tag gene into an expression vector already containing your gene of interest.

Expression

Expression can be analyzed by running samples on an SDS-PAGE gel. For SNAP-tag fusion proteins you should see a band of molecular weight approximately 20 kDa bigger than your protein of interest. SNAP-tag fusion proteins are most easily visualized on a gel using fluorescent imaging after labeling with 10 µM SNAP Vista Green (NEB #S9147). If no protein can be seen at the expected molecular weight, a Western blot with an antibody against your protein or the Anti-SNAP-tag Antibody (NEB #P9310) should give higher sensitivity detection.

In general we have not experienced problems expressing SNAP-tag protein fusions. However if your fusion gene does not appear to be expressed, try expressing the SNAP-tag alone as a positive control, using cells transformed with the pSNAP-tag(T7)-2 expression plasmid. You should clearly see a band at approximately 20 kDa in induced cells representing SNAP26b protein. If you do not see expression, check your expression conditions: suitable host strain, appropriate selection (ampicillin for pSNAP-tag(T7)-2), freshly grown colony and correct induction conditions.

If the SNAP-tag alone is expressed but your fusion protein is not, then there are a variety of possible causes:

- It is possible that this fusion protein may be toxic for bacteria. Some rare proteins are extremely toxic when expressed in host bacteria and will not show expression in most *E. coli* based expression systems. It is difficult to troubleshoot such instances, but the use of a highly repressed, stringently induced promoter (tightly regulated T7 expression hosts such as T7 Express[®] (NEB #C3016) or T7 Express *lysY*[®] (NEB #C3013) may help. Signs of host-cell toxicity could be inhibition of bacterial growth, or even cell lysis after induction.

- The fusion protein may be unstable in *E. coli*. There are several reasons why heterologous proteins may be unstable in *E. coli*, such as incomplete folding and rapid protease degradation.
- Some recombinant proteins cannot be expressed in *E. coli*, in which case use of another expression system should be considered.

If the expression of a soluble protein is weak due to toxicity or instability, growing the bacterial culture to an OD₆₀₀ of 1.0 to 1.5 and then using a short induction time may improve the results.

Recombinant proteins in *E. coli* frequently partition partly or totally into inclusion bodies. This may result from over-expression and may be caused by limited protein solubility or represent the aggregation and accumulation of improper folding intermediates. SNAP-tag can usually be found in both the soluble and insoluble cell fractions. Generally sufficient SNAP-tag is present in soluble fractions for subsequent purification.

References

1. Keppler, A. et al. (2003) *Nat. Biotechnol.* 21, 86.
2. Gautier, A. et al. (2008) *Chem. Biol.* 15, 128.
3. Keppler, A. et al. (2004) *Proc. Natl. Acad. Sci. USA* 101, 9955.
4. Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.* 219, 45.



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7,888,090 (Mutants of O⁶-Alkylguanine-DNA-Alkyltransferases)
8,163,479 Specific Substrates for O⁶-Alkylguanine-DNA-Alkyltransferases)
8,178,314 (Pyrimidines Reacting With O⁶-Alkylguanine-DNA-Alkyltransferases)
PCT/EP2007/057597 (Labeling of Fusion Proteins with Synthetic Probes)
EP07117800 (Drug Delivery)
EP07117802 (Drug Delivery)
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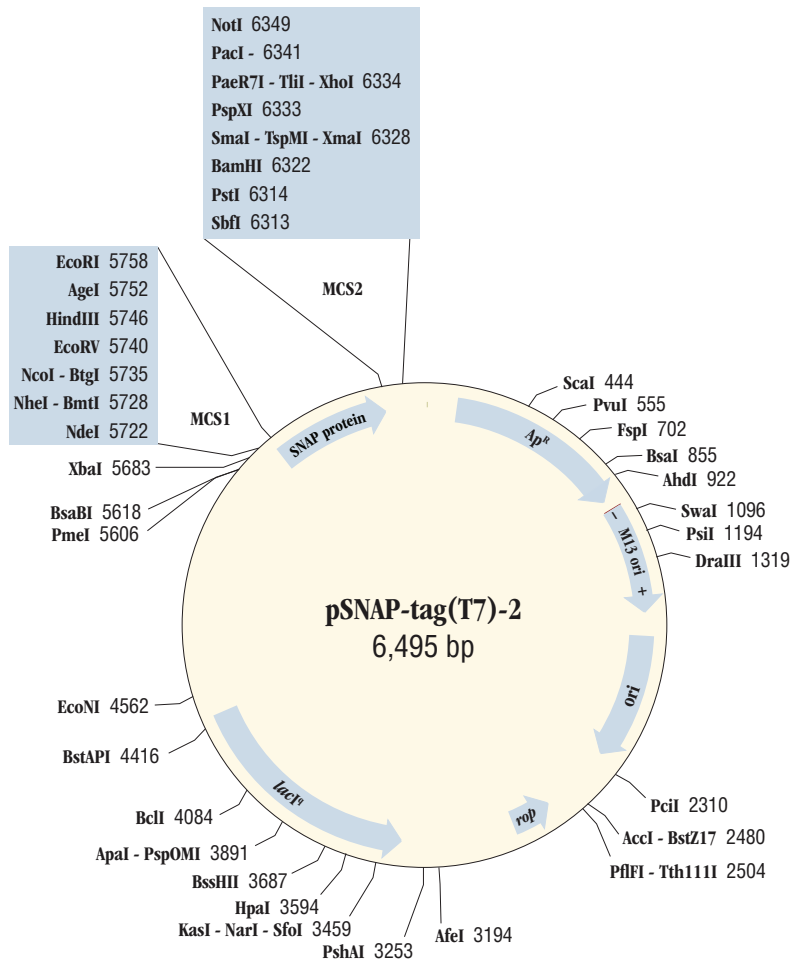
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Plasmid Map of pSNAP-tag(T7)-2



Cloning Region of pSNAP-tag(T7)-2

Unique restriction sites in the regions flanking the *SNAP26b* gene are displayed above the coding strand. This map and the maps for the control plasmids can be downloaded at www.neb.com

Companion Products:

T7 Express Competent *E. coli*
 #C2566H 20 x 0.05 ml
 #C2566I 6 x 0.2 ml

T7 Express *lysY* Competent *E. coli*
 #C3010H 20 x 0.05 ml
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T7 Express ^{fl} Competent *E. coli*
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5632   T7 Promoter          lac operator          XbaI
GAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCTCTAGAAAT

5692   RBS              NdeI  NheI    NcoI  EcoRV  HindIII
AATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTAGCACCATGGATATCAAGCTT
                                     M A S T M D I K L

5752   AgeI  EcoRI
ACCGGTGAATTCGCTATGGACAAAGATTGCGAAATGAAACGTACCACCCTGGATAGCCCG
T G E F A M D K D C E M K R T T L D S P

5812   SNAP26b
CTGGGCAAACCTGGAACCTGAGCGGCTGCGAACAGGGCTGCATGAAATTAACCTGCTGGGT
L G K L E L S G C E Q G L H E I K L L G

5872   AAAGGCACCAGCGCGCCGATGCGGTTGAAGTTCGCGCCCGCGCCGCTGCTGGGTGGT
K G T S A A D A V E V P A A V L G G

5932   CCGGAACCGCTGATGCAGGCGACCGCTGGCTGAACGCGTATTTTCATCAGCCGGAAGCG
P E P L M Q A T A W L N A Y F H Q P E A

5992   ATTGAAGAATTTCCGGTTCGCGCTGCATCATCCGGTGTTCAGCAGGAGAGCTTTACC
I E E F P V P A L H H P V F Q Q E S F T

6052   CGTCAGGTGCTGTGAAACTGCTGAAAGTGGTAAATTTGGCGAAGTGATTAGCTATCAG
R Q V L W K L L K V V K F G E V I S Y Q

6112   CAGCTGGCGCCCTGGCGGTAATCCGGCGGCCACCGCCCGTTAAACCGCGCTGAGC
Q L A A L A G N P A A T A A V K T A L S

6172   GGTAACCCGGTGCCGATTCTGATTCCGTGCCATCGTGTGGTTAGCTCTAGCGGTGCGGTT
G N P V P I L I P C H R V V S S S G A V

6232   GCGGTTATGAAGGTGGTCTGGCGGTGAAAGAGTGGCTGCTGGCCATGAAGGTCATCGT
G G Y E G G L A V K E W L L A H E G H R

6292   SbfI/PstI  BamHI  SmaI  XhoI  PacI
CTGGGTAAACCGGGTCTGGGACCTGCAGGCGGATCCCCGGGCTCGAGGTTAATTAAGCG
L G K P G L G P A G G S P G L E V N *

6352   NotI
GCCGCATTGATCCGGCTGCTAACAAAGCCC GAAAGGAAGCTGAGTTGGCTGCTGCCACCG

6412   CTGAGCAATAACTAGCATAACCCC
    
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