

## Data Sheet

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# pASK-IBA1 3plus

Cat. No. : 2-1412-000

Lot No.: 1412-

Last date of revision  
May 10

Version 1412-8

<b>Description</b>	Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be localized in the cytoplasm.
<b>Affinity tag</b>	<i>Strep-Tactin</i> <sup>®</sup> affinity tag ( <i>Strep-tag II</i> <sup>®</sup> ) for the purification of recombinant protein. The affinity tag is fused to the N-terminus of the recombinant protein and can be removed by cleavage with Thrombin. The cleavage is enhanced due to a "kinker" site <sup>1)</sup> (spacer).
<b>Bacterial Expression</b>	Expression is induced upon addition of 200 µg anhydrotetracycline (order no.: 2-0401-001; 2-0401-002) per 1 liter <i>E. coli</i> shaking culture ( $A_{550} = 0.5$ ).
<b>Expression strain</b>	Any <i>E. coli</i> strain. The <i>tet</i> -promoter works independently from the genetic background of <i>E. coli</i> .
<b>Resistance</b>	Ampicillin
<b>Form</b>	5 µg, dissolved in 10 mM Tris/HCl pH 8.0, 1 mM EDTA; 20 µl
<b>Concentration</b>	250 ng/µl
<b>Storage</b>	4 °C for frequent usage, -20 °C for long-term storage

1) Hakes, J.D. & Dixon, J.E. (1992): *Anal. Biochem.* 202, 293-298.

## For research use only

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## Multiple Cloning Site of pASK-IBA13plus

```

1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTGTGACTCTATCATTGATAGAGTTAATTTACCACTCCCTATC 79
                                     forward primer

80     AGTGATAGAGAAAAGTGAATGAATAGTTCGACAAAAATCTAGAATAATTTTGTTTAACTTTAAGAAGGAGATATACAA 159
                                     XbaI

      link      Streptag      kinker      thrombin      R P R
      M A S W S H P Q F E K S G G G G G L V P R G S R D R G
160    ATGGCTAGCTGGAGCCACCCGAGTTCGAAAAATCTGGTGGTGGTGGTCTGGTTCCGCGTGGGtccCGAGACCGCGG 239
      NheI                                     BsaI
                                               PshAI
                                               SacII

      S R I R A R Y P G I P R G R P A G G P W S L I S N *
      V P N S S S V P G D P S R S T C R G T M V S D I *
      P E F E L G T R G S L E V D L Q G D H G L *

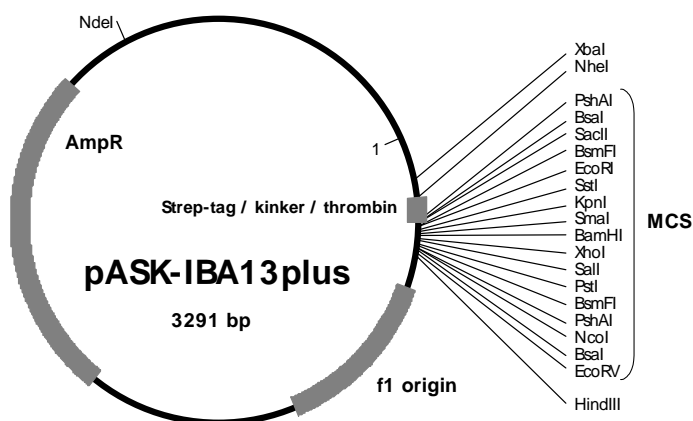
240    TCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCTgataTCTAACTAAGC 319
      EcoRI      KpnI      BamHI      SalI PstI BsmFI BsaI EcoRV HindIII
BsmFI      SstI      SmaI      XhoI      PshAI
                                               NcoI

320    TTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTTGTCTGCCGTTTACCGCTACTGCGTCACGGATCT 399
                                     reverse primer
  
```

**Please note:** Restriction enzymes in bold cut twice. The *BsaI* sites (isoschizomer of *Eco31I*) at each end of the multiple cloning site are useful for precise and oriented insertion of the recombinant gene by one cleavage reaction only. The "link" contains a restriction site which can be used e.g. for subcloning the recombinant gene into pEXPR-IBA vectors for mammalian expression.

## Features of pASK-IBA13plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
Strep-tag	160	192
kinker	193	210
thrombin cleavage site	211	228
multiple cloning site	229	305
reverse primer binding site	373	389
f1 origin	402	840
AmpR resistance gene	989	1849
Tet-repressor	1859	2484
Col E1 origin	2637	3225



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNC TCC <sup>(N<sub>20</sub>)</sup> NNN NNN...	Forward: 5'- GAGTTAATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA <sup>(N<sub>20</sub>)</sup> NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'