

## Data Sheet

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# pASK-IBA43plus

Cat. No. : 2-1443-000

Lot No.: 1443-

Last date of revision  
**May 10**

Version 1443-9

<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>	The recombinant protein will contain two affinity tags: <ol style="list-style-type: none"> <li><b>1)</b> <i>Strep-Tactin</i><sup>®</sup> affinity tag (<i>Strep-tag II</i><sup>®</sup>) for the purification of recombinant protein via <i>Strep-Tactin</i> resins. The <i>Strep-tag</i> is fused to the C-terminus of the recombinant protein.</li> <li><b>2)</b> 6xHistidine-tag for the purification of recombinant protein via Ni-NTA resins. The 6xHistidine-tag is fused to the N-terminus of the recombinant protein.</li> </ol>
<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

## For research use only

### Important licensing information

This product is based on *Strep-tag*, 6xHistidine-tag and *tet* promoter technologies covered by intellectual property (IP) rights and on completion of the sale IBA grants respective Limited Use Label Licenses to purchaser. IP rights and Limited Use Label Licenses for said technology are further described and identified at <http://www.iba-go.com/patents.html> or upon inquiry at [info@iba-go.com](mailto:info@iba-go.com) or at IBA GmbH, Rudolf-Wissell-Str. 28, 37079 Göttingen, Germany. By use of this product the purchaser accepts the terms and conditions of all applicable Limited Use Label Licenses.

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## Multiple Cloning Site of pASK-IBA43plus

```

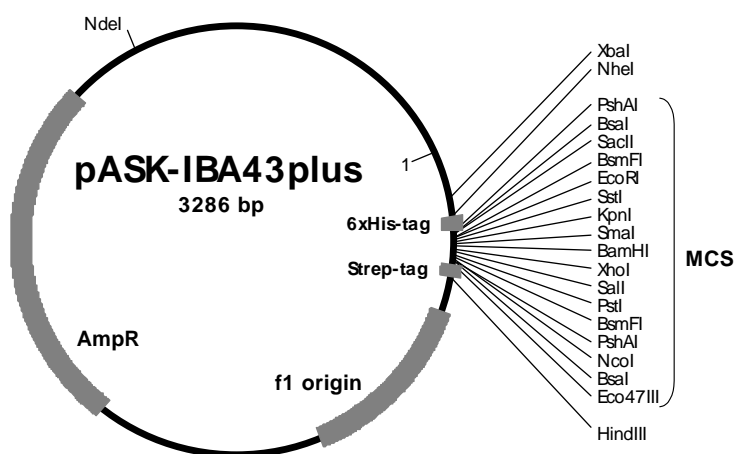
1      CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATC 79
                                     forward primer
80     AGTGATAGAGAAAAGTGAATGAATAGTTCGACAAAAATCTAGAAATAATTTGTTTAACTTTAAGAAGGAGATATACAA 159
                                     XbaI
        link      6xHistidine-tag
        M A S R G S H H H H H H G A G D R G P E F E L G T R G
160    ATGGCTAGCAGAGGATCGCATCACCATCACCATCACGGggccGGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGG 239
        NheI      BsaI      BsmFI      SstI      KpnI      BamHI
                  PshAI      EcoRI      SmaI
                  SacII

        link      Strep-tag
        S L E V D L Q G D H G L S A W S H P Q F E K *
240    ATCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCAGcgcTTGGAGCCACCCGAGTTCGAAAAATAATAAGCTTGAC 319
        XhoI SalI PstI BsmFI BsaI Eco47III HindIII
                  PshAI
                  NcoI
320    CTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTTGCTGCGTTTACCGCTACTGCGTCACGGATCTCCACG 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-IBA43plus

	from bp	to bp
promoter	37	72
forward primer binding site	57	76
6xHistidine-tag	160	195
multiple cloning site	196	282
Strep-tag	283	312
reverse primer binding site	368	384
f1 origin	397	835
AmpR resistance gene	984	1844
Tet-repressor	1854	2477
Col E1 origin	2630	3218



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNGC GCC <sup>(N<sub>20</sub>)</sup> NNN NNN...	Forward: 5'- GAGTTATTTTACCACTCCCT -3'
Reverse: 5'- NNNNNNGGTCTCNGC GCT <sup>(N<sub>20</sub>)</sup> NNN NNN...	Reverse: 5'- CGCAGTAGCGGTAAACG -3'