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# Data Sheet

# pASK-IBA7C

Cat. No.: 2-1326-000
Lot No.: 1326-

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| | |
|---|---|
| **Description** | Expression plasmid. The expression cassette is under transcriptional control of the tetracycline promoter/operator. The expressed recombinant protein will be localized in the cytoplasm. |
| **Affinity tag** | Strep-Tactin® affinity tag (Strep-tag®II) 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 factor Xa. |
| **Bacterial Expression** | Expression is induced upon addition of 200 µg anhydrotetracycline (order no.: 2-0401-001; 2-0401-002) per 1 liter *E. coli* shaking culture ($A_{550}$ = 0.5). |
| **Expression strain** | Any *E. coli* strain. The *tet*-promoter works independently from the genetic background of *E. coli*. |
| **Resistance** | Chloramphenicol<br>**Note:** The CamR resistance gene codes for homotetrameric chloramphenicol acetyltransferase (MW of the monomer = 26.6 kDa) which is predominantly expressed in the cytosol of *E.coli* transformed with this plasmid |
| **Form** | 5 µg, dissolved in 20 µl TE buffer, pH 8,0: 10 mM Tris-HCl, 1 mM EDTA |
| **Concentration** | 250 ng/µl |
| **Stability** | 12 months after shipping |
| **Storage** | recommended: 2-8 °C for frequent usage, -20 °C for long-term storage |
| **Shipping** | room temperature |
| **Hazards** | Product is not classified as hazardous according to (EC) No 1272/2008 [CLP].<br>A Material Safety Data Sheet is provided. |

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**For research use only**

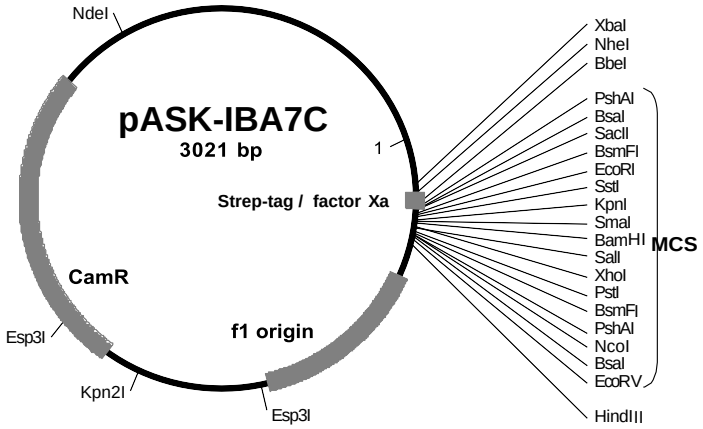
## Multiple Cloning Site of pASK-IBA7C

```
1       CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA     80
                                                                 forward primer

                                                                    link
                                                                 M A S W S H P
81      GTGATAGAGAAAAGTGAAATGAATAGTTCGACAAAAATCTAGATAACGAGGGCAAAAAATGGCTAGCTGGAGCCACCCGC     160
                                             XbaI                    NheI

                                          R P R S R I R A R Y P G I P R G R P
      Strep-tag®II   factor Xa   E T  A  V  P  N  S  S  S  V  P  G  D  P  S  R  S  T  C
        Q F E K  I E G R R D R G P E F E L G T R G S L E V D  L
161     AGTTCGAAAAAATCGAAGGgcgcCGAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTG     240
                        BbeI  BsaI     BsmFISstI  KpnI      BamHI      SalI          PstI
                        EheI    PshAI       EcoRI           SmaI       XhoI
                        KasI      SacII
                        NarI

        A G  G  P  W  S  L  I  S  N  *
          R G T M V S D I  *
         Q G D H G L  *
241     CAGGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTT     320
            BsmFI   BsaI   EcoRV      HindIII
              PshAI
                NcoI

321     TGTCTGCCCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTT     400
                reverse primer
```

**Please note:** Restriction enzymes in bold cut twice. The *Bsa*I sites (isoschizomer of *Eco31*I) 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-IBA7C

| | from bp | to bp |
|---|---|---|
| promoter | 37 | 72 |
| forward primer binding site | 57 | 76 |
| Strep-tag®II | 139 | 171 |
| factor Xa cleavage site | 172 | 183 |
| multiple cloning site | 184 | 260 |
| reverse primer binding site | 328 | 344 |
| f1 origin | 357 | 795 |
| CamR resistance gene | 917 | 1576 |
| Tet-repressor | 1589 | 2212 |
| Col E1origin | 2365 | 2953 |




| **Cloning primers for the precise cloning using *Bsa*I or *Eco31*I** | **Sequencing primers:** |
|---|---|
| Forward: 5'- NNNNNNGGTCTCNG CGC $(N_{20})$ NNN NNN... | Forward: 5'- GAGTTATTTTACCACTCCCT -3' |
| Reverse: 5'- NNNNNNGGTCTCNTA TCA $(N_{20})$ NNN NNN... | Reverse: 5'- CGCAGTAGCGGTAAACG -3' |