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Data Sheet

pASK-IBA7C

Cat. No.: 2-1326-000

Version: 11.0
Revision Date: 11.06.2021

| | |
|-----------------------------|---|
| 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-tag®II 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 per 1 liter <i>E. coli</i> shaking culture ($A_{550} = 0.5$). |
| Expression strain | Any <i>E. coli</i> strain. The <i>tet</i> -promoter works independently from the genetic background of <i>E. coli</i> . |
| Resistance | Chloramphenicol Note: The Cam ^R resistance gene codes for homotetrameric chloramphenicol acetyltransferase (MW of the monomer = 26.6 kDa) which is predominantly expressed in the cytosol of <i>E. coli</i> 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]. A Material Safety Data Sheet is provided. |

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Multiple Cloning Site of pASK-IBA7C

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1  CCATCGAATGGCCAGATGATTAATTCCTAATTTTTGTTGACACTCTATCATTGATAGAGTTATTTTACCACTCCCTATCA  80
                                     forward primer
                                     link Strep-tag®II
                                     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
                                     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 AGTTCGAAAAAATCGAAGGgCgCgCAGACCGCGGTCCCGAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTCGACCTG  240
                                     BbeI  BsaI    BsmFI   SstI  KpnI    BamHI    SalI  PstI
                                     EheI  PshAI   EcoRI           SmaI    XhoI
                                     KasI    SacII
                                     NarI

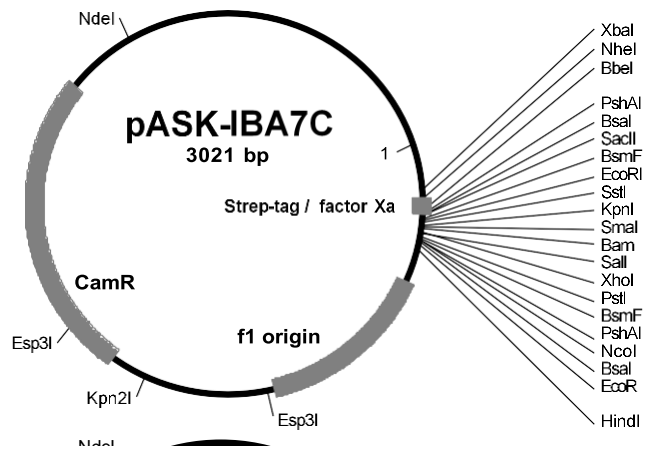
A G G P W S L I S N *
R G T M V S D I *
Q G D H G L *
241 CAGGGGGACCATGGTCTCTgataTCTAACTAAGCTTGACCTGTGAAGTGAAAAATGGCGCACATTGTGCGACATTTTTTT  320
                                     BsmFI  BsaI  EcoRV      HindIII
                                     PshAI
                                     NcoI

321 TGTCTGCCGTTTACCGCTACTGCGTCACGGATCTCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGGGTGTGGTGGTT  400
                                     reverse primer
  
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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 for subcloning.

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 |
| ColE1 origin | 2365 | 2953 |



| Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i> | Sequencing primers: |
|--|---------------------------------------|
| Forward: 5'- NNNNNNGGTCTCNGC GCC ^(N₂₀) NNN NNN... | Forward: 5'- GAGTTATTTTACCACTCCCT -3' |
| Reverse: 5'- NNNNNNGGTCTCNTA TCA ^(N₂₀) NNN NNN... | Reverse: 5'- CGCAGTAGCGGTAAACG -3' |