

Data Sheet

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pPR-IBA2

Cat. No. : 2-1391-000

Lot No.: 1391 -

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May 10

Version 1391-6

Description	Expression plasmid for either <i>in vitro</i> transcription/translation or bacterial expression. The expression cassette is under transcriptional control of the strong bacteriophage T7 promoter.
<i>In vitro</i> Expression	T7 promoter-based expression; T7 RNA polymerase has to be included in the <i>in vitro</i> transcription/translation system
Bacterial Expression	T7 promoter-based expression; T7 RNA polymerase is produced in <i>E. coli</i> BL21 (DE3).
Affinity tag	<i>Strep</i> -Tactin® affinity tag (<i>Strep</i> -tag II®) for the purification of recombinant protein. The affinity tag is fused to the N-terminus of the recombinant protein
Resistance	Ampicillin
Form	Dissolved in 10 mM Tris/HCl pH 8.0, 1 mM EDTA; 20 µl
Concentration	250 ng/µl
Storage	4 °C for frequent usage; -20 °C for long-term storage

For research use only

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Multiple Cloning Site of pPR-IBA2

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1      GATCTCGATCCCGCAAATTAATACGACTCACTATAGGGAGGCCACAACGGTTTCCCTCTAGAAATAATTTGTTTAACT      80
          forward primer                                XbaI

          D R G P E F E
          Strep-tag      link      R P R S R I R
          M A S W S H P Q F E K G A E T A V P N S

81     TTAAGAAGGAGATATACATATGGCTAGCTGGAGCCACCCGAGTTCGAAAAAGgcgcCGAGACC GCGGTCCCGAATTCGA      160
          NdeI  NheI                                BbeI  BsaI  BsmFI  SstI
          EheI  PshAI  EcoRI
          KasI  SacII
          NarI

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

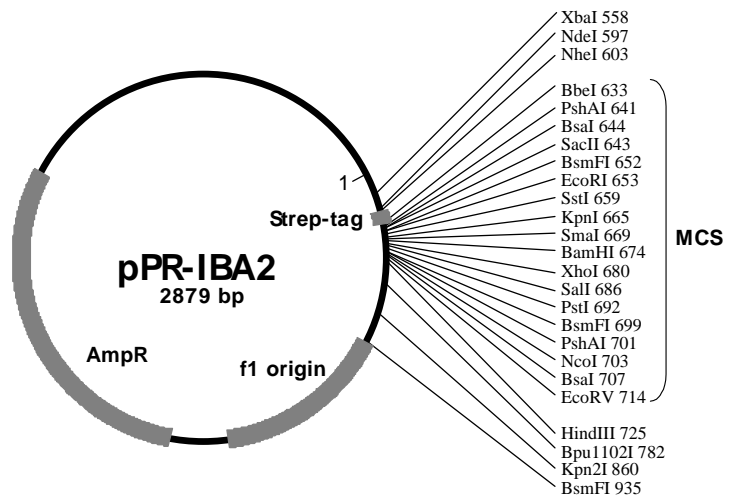
161    GCTCGGTACCCGGGATCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCTgataCTAACTAAGCTTGATCCGGCTG      240
          KpnI  BamHI  SalI  PstI  BsmFI  BsaI  EcoRV  HindIII
          SmaI  XhoI
          PshAI
          NcoI

241    CTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAA      320
          reverse primer
          Bpu102I
  
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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 e.g. for subcloning the recombinant gene into pASK-IBA vectors for prokaryotic expression or pEXPR-IBA vectors for mammalian expression.

Features of pPR-IBA2

	from bp	to bp
forward primer binding site	20	39
Strep-tag	100	132
multiple cloning site	133	214
reverse primer binding site	277	296
f1 origin	438	876
AmpR resistance gene	1024	1883
Col E1 origin	2061	2733



Cloning primers for the precise cloning using *BsaI* or *Eco31I*

Forward: 5'- NNNNNNGGTCTCNGC GCC ^(N₂₀) NNN NNN...

Reverse: 5'- NNNNNNGGTCTCNTA TCA ^(N₂₀) NNN NNN...

Sequencing primers:

Forward: 5'- TAATACGACTCACTATAGGG -3'

Reverse: 5'- TAGTTATTGCTCAGCGGTGG -3'