

Data Sheet

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pPR-IBA 1

Cat. No. : 2-1390-000

Lot No.: 1390 -

Last date of revision
May 10

Version 1390-6

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	Strep-Tactin® affinity tag (Strep-tag II®) for the purification of recombinant protein. The affinity tag is fused to the C-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-IBA1

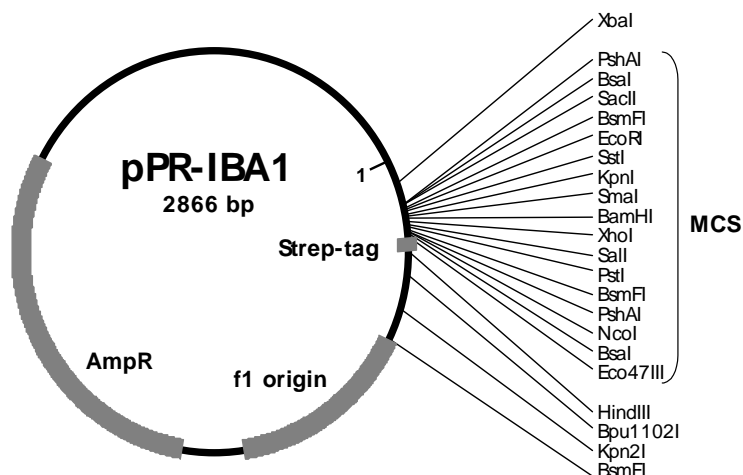
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1      GATCTCGATCCC GCGAAATTAATACGACTCACTATAGGGAGGCCACAACGGTTTCCTCTAGAAATAATTTGTTTAACT  80
      forward primer                                     XbaI
      M G D R G P E F E L G T R G S L E V D L
81     TTAAGAAGGAGATATACAAatgGGAGACCGCGGTCCC GAATTCGAGCTCGGTACCCGGGGATCCCTCGAGGTTCGACCTGC  160
      BsaI      BsmFI      SstI KpnI      BamHI      SalI PstI
      PshAI      EcoRI      SmaI      XhoI
      SacII
      link      Strep-tag
      Q G D H G L S A W S H P Q F E K *
161    AGGGGACCATGGTCTCAGcgcTTGGAGCCACCCGAGTTCGAAAAATAATAAGCTTGATCCGGCTGCTAACAAAGCCCC  240
      BsmFI BsaI Eco47III      HindIII
      PshAI
      NcoI
241    AAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGG  320
      reverse primer
      Bpu1102I
  
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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-IBA1

	from bp	to bp
forward primer binding site	20	39
multiple cloning site	100	183
Strep-tag	184	213
reverse primer binding site	264	283
f1 origin	425	863
AmpR resistance gene	1011	1870
Col E1 origin	2048	2720



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNA ATG ^(N₁₇) NNN NNN...	Forward: 5'- TAATACGACTCACTATAGGG -3'
Reverse: 5'- NNNNNNGGTCTCNGC GCT ^(N₂₀) NNN NNN...	Reverse: 5'- TAGTTATTGCTCAGCGGTGG -3'

* The ATG start codon is already included