



Data Sheet

pPR-IBA2

Cat. no. : 2-1391-000

Lot no.: 1391 -

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Version 1391-4

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 μ l
Concentration	250 ng/ μ l
Storage	4 °C for frequent usage; -20 °C for long-term storage

For research use only

Strep-tag® technology for protein purification and detection is covered by US patent 5,506,121, UK patent 2272698 and French patent 93 13 066; the tetracycline promoter based expression system is covered by US patent 5,849,576 and *Strep*-Tactin® is covered by US patent 6,103,493. Further patent applications are pending world-wide. Purchase of reagents related to these technologies from IBA provides a license for non-profit and in-house research use only. Expression or purification or other applications of above mentioned technologies for commercial use require a separate license from IBA. A license may be granted by IBA on a case-by-case basis, and is entirely at IBA's discretion. Please contact IBA for further information on licenses for commercial use. *Strep*-tag® and *Strep*-Tactin® are registered trademarks of IBA GmbH.

Multiple Cloning Site of pPR-IBA2

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1      GATCTCGATCCCGCAAATTAATACGACTCACTATAGGGAGGCCACAACGGTTTCCTCTAGAAATAATTTGTTTAACT  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     TTAAGAAGGAGATATACATATGGCTAGCTGGAGCCACCCGAGTTCGAAAAAGgcgcCGAGACCGGGTCCCGAATTCGA  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 *

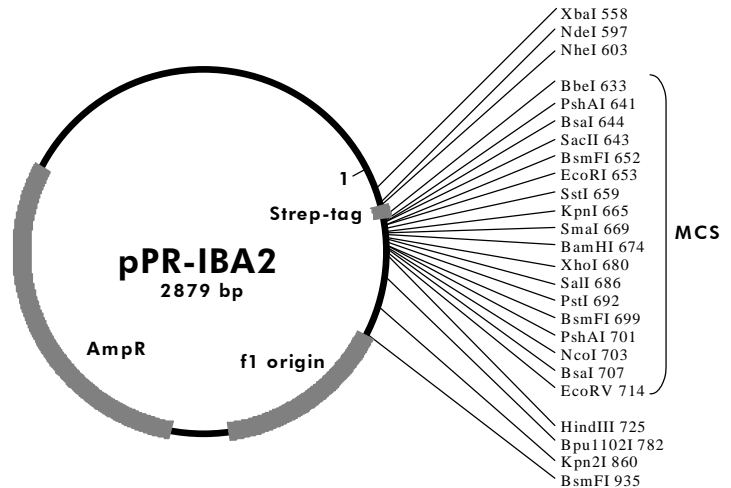
161    GCTCGGTACCCGGGGATCCCTCGAGGTCGACCTGCAGGGGACCATGGTCTCTgataCTAACTAAGCTTGATCCGGCTG  240
           KpnI   BamHI   SalI  PstI   BsmFI  BsaI  EcoRV   HindIII
           SmaI   XhoI
           PshAI
           NcoI

241    CTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAA  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-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



Cloning primers for the precise cloning using <i>BsaI</i> or <i>Eco31I</i>	Sequencing primers:
Forward: 5'- NNNNNNGGTCTCNGC GCC (N ₂₀) NNN NNN...	Forward: 5'- TAATACGACTCACTATAGGG -3'
Reverse: 5'- NNNNNNGGTCTCNTA TCA (N ₂₀) NNN NNN...	Reverse: 5'- TAGTTATTGCTCAGCGGTGG -3'