MRC PPU REAGENTS

Standard Operating Procedure

Preparation of BORA [1 - 559]

Enzyme description:- BORA [1 – 559]

Clone number:- DU 15717

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 88, 949.46 daltons
Average Mass 89, 006.20 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.04

Purity:- >75 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

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Clone Data Sheet

BORA [1 – 559]

Protein BORA [1 – 559]

Clone number DU 15717

Species Human

Accession number Q6PGQ7-1

Tags N-terminal GST

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPGIPGSTRA
AA**MGDVKESKMQITPETPGRIPVLNPFESPSDYSNLHEQTLASPSVFK**
STKLPTPGKFRWSIDQLAVINPVEIDPEDIHRQALYLSHSRIDKDVED
KRQKAIEEFFTKDVIVPSPWTDHEGKQLSQCHSSKCTNINSDSPVGKK
LTIHSEKSDAACQTLLSLPVDFNLENILGDYFRADEFADQSPGNLSSS
SLRRKLFLDGNGSISDSLPSASPGSPHSGVQTSLEMFYSIDLSPVKCR
SPLQTPSSGQFSSSPIQASAKKYSLGSITSPSPISSPTFSPIEFQIGE
TPLSEQRKFTVHSPDASSGTNSNGITNPCIRSPYIDGCSPIKNWSPMR
LQMYSGGTQYRTSVIQIPFTLETQGEDEEDKENIPSTDVSSPAMDAAG
IHLRQFSNEASTHGTHLVVTAMSVTQNQSSASEKELALLQDVEREKDN
NTVDMVDPIEIADETTWIKEPVDNGSLPMTDFVSGIAFSIENSHMCMS
PLAESSVIPCESSNIQMDSGYNTQNCGSNIMDTVGAESYCKESDAQTC
EVESKSQAFNMKQDHTTQRCWMKTASPFQCSSP

Native sequence Amino acids M1 – P559 (end) of human BORA.
Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 – 228

Cloning sites *Not*1 sites of pGEX6P-2

Nucleotide Sequence Of Insert

gcggccgcgATGGGAGATGTCAAGGAATCAAAGATGCAAATAACACCAGA
AACTCCAGGAAGGATCCCTGTTTTAAATCCTTTTGAAAGTCCTAGTGATT
ATTCTAATCTCCATGAACAAACTCTCGCCAGTCCTTCTGTTTTTAAATCA
ACAAAATTACCAACTCCAGGGAAATTTAGATGGTCTATTGATCAACTAGC
TGTAATAAATCCTGTAGAAATAGACCCAGAAGATATTCATCGTCAAGCTT
TATACTTAAGTCATTCTCGAATAGATAAAGATGTGGAAGACAAAAGACAA
AAAGCCATTGAAGAGTTTTTCACTAAAGATGTCATCGTACCCTCTCCTTG
GACTGATCATGAAGGGAAACAGCTTTCACAATGTCATTCCAGTAAATGCA
CTAACATAAATAGTGACTCTCCAGTTGGAAAAAAGCTGACCATTCATTCT
GAGAAAAGCGATGCTGCTTGTCAGACATTGCTGTCTCTTCCTGTGGATTT
TAATTTAGAAAATATATTAGGTGACTATTTTAGAGCTGATGAATTTGCAG
ATCAATCTCCTGGAAACCTCAGTTCTTCATCCCTCAGAAGAAAGCTGTTT
TTAGATGGGAACGGAAGCATCTCCGACTCCTTACCTTCGGCTTCTCCCGG
AAGTCCTCACAGTGGTGTTCAAACATCACTAGAGATGTTTTATTCAATAG
ATTTGTCTCCTGTAAAGTGTAGGAGCCCCTTGCAGACACCAAGTTCGGGG
CAGTTTTCTTCTAGCCCTATTCAGGCTAGTGCAAAAAAATACAGCTTGGG
AAGCATAACTAGTCCTTCGCCTATTTCTTCACCCACTTTCTCACCAATTG
AATTTCAGATAGGAGAGACTCCACTCTCAGAACAAAGGAAGTTTACTGTT
CATTCTCCTGATGCTTCATCTGGAACAAATTCTAATGGGATAACTAATCC
GTGTATCAGAAGTCCTTATATAGATGGCTGCTCGCCAATTAAAAATTGGT
CTCCTATGAGACTTCAGATGTATAGTGGTGGTACTCAGTATCGGACCTCA
GTGATTCAGATACCTTTTACTCTTGAGACTCAAGGTGAAGATGAGGAAGA
TAAAGAGAATATTCCTTCCACAGATGTCTCATCACCCGCCATGGATGCTG
CTGGAATACACCTACGGCAGTTTAGTAATGAGGCTTCTACCCATGGTACA
CATTTGGTTGTGACTGCCATGTCTGTTACACAAAATCAGTCCAGTGCTTC
TGAGAAAGAATTAGCACTGTTGCAGGATGTTGAAAGGGAGAAAGACAATA
ACACTGTGGATATGGTTGATCCTATAGAGATAGCAGATGAGACCACTTGG
ATTAAGGAGCCGGTTGATAATGGCAGTTTACCCATGACTGATTTTGTAAG
TGGCATTGCCTTCAGTATTGAAAACTCTCATATGTGCATGTCACCTCTTG
CTGAAAGCAGTGTCATTCCTTGTGAAAGCAGTAACATTCAGATGGATAGT
GGCTATAATACGCAGAATTGTGGAAGCAATATTATGGATACAGTTGGGGC
AGAAAGTTACTGCAAAGAAAGTGATGCACAAACATGTGAAGTTGAGAGTA
AATCTCAAGCATTTAATATGAAGCAAGACCACACAACACAGAGGTGTTGG
ATGAAAACAGCAAGCCCTTTTCAATGCAGCAGTCCAtaggcggccgc