

14 April 2020

QC Document: RON-wt, RON- Δ 160 and RON- Δ 155

Constructs

>RON_wt-TEV-FLAG-10His_pTT5

MELLPLPQSFLLLLLPAKPAAGEDWQCPRTPYAASRDFDVKYVVPFSFAGGLVQAMVTYEGDRNESAVFVAIR
NRLHVLGPD LKSVQSLATGPAGDPGCQTCAACGPGPHGPPGDTDTKVLVLDPALPALVSCGSSLQGRCLHDLE
PQGTAVHLAAPACLFSAHHNRPD DCPDCVASPLGTRVTVVEQGQASYFYVASSLDAAVAASFSPRSVSIRRLKAD
ASGFAPGFVALSVLPKHLVSYSIEYVHSFHTGAFVYFLTVQPASVTDDPSALHTRLARLSATEPELGDYRELVLDCRF
APKRRRRGAPEGGQPYVLRVAHSAPVGAQLATELSIAEGQEVLFVGVFTGKDGPGVGPNSVVCAPIDLLDTL
IDEGVERCCESPVHPGLRRGLDFFQSPSFCPNPPGLEALSPNTSCRHFPLLVS SFSRVDLFNGLLGPVQVTALYVTR
LDNVTVAHMGTM DGRILQVELVRSLNYLLYVS N FSLGDSGQPVQRDVSRLGDHLLFASGDQVFQVPIQGP GCR
HFLTCGRCLRAWHFMGCGWCGNMCGQQKECPGSWQQDHCPPKLTEFHPHSGPLRGSTRLTLCGSNFYLHPS
GLVPEGTHQVTVGQSPCRPLPKDSSKL RPVPRKDFVEEFECLEPLGTQAVGPTNVSLTVTNMPPGKHFRVDGTS
VLRGFSFMEPVLIQVPLFGPRAGGTCLTLEGQSLSVGTSRAVLVNGTECLLARVSEGQLLCATPPGATVASVPLSL
QVGGAQVPGSWTFQYREDPVVLSISPNCGYINSHITICGQHLSAWHLVLSFHDGLRAVESRCERQLPEQQLCRL
PEYVVRDPQGWVAGNLSARGDGAAGFTLPGFRFLPPPHPPSANLVPLKPEEHAIKFEYIGLGAVADCVGINVTVG
GESQCHEFRGDMVVCPLPSSLQLGQDGAPLQVCVDGECHILGRVVRPGPDGVPQSTLLGILLPLLLLVAALATAL
VFSYWWRRKQLVLPNNLDLASLDQTAGATPLILYSGSDYRSGALPAIDGLDSTTCVHGASFSDSEDESCVPLLR
KESIQLRDLDSALLAEVKDVLIPHERVVTHSDRVIGKGHFGVVYHGEYIDQAQNRICAIKSLSRITEMQQVEAFRL
EGLLMRGLNHPNVLALIGIMLPPEGLPHVLLPYMCHGDLQFIRSPQRNPTVKDLISFGLQVARGMEYLAEQKFV
HRDLAARNCMLDESFTVKVADFG LARDILDREYYSVQQRHARLPVKWMALES LQTYRFTTKSDVWSFGVLLW
ELLTRGAPPYRHIDPFDLTHFLAQGRRLPQPEYCPDSLYQVMQQCWEADPAVRPTFRVLVGEVEQIVSALLGDHY
VQLPATYMN LGPSTHEMNVRPEQPQFSPMPGNVRRRPRPLSEPPRPTENLYFQGS DYKDDDDKG HHHHHHHH
HH

Number of amino acids: 1427

Molecular weight: 155603.87

Theoretical pI: 6.10

Extinction coefficients:

Extinction coefficients are in units of $M^{-1} cm^{-1}$, at 280 nm measured in water.

Ext. coefficient 126400

Abs 0.1% (=1 g/l) 0.812, assuming all pairs of Cys residues form cystines

>RON_ Δ 160-TEV-FLAG-10His_pTT5

MELLPLPQSFLLLLLPAKPAAGEDWQCPRTPYAASRDFDVKYVVPFSFAGGLVQAMVTYEGDRNESAVFVAIR
NRLHVLGPD LKSVQSLATGPAGDPGCQTCAACGPGPHGPPGDTDTKVLVLDPALPALVSCGSSLQGRCLHDLE
PQGTAVHLAAPACLFSAHHNRPD DCPDCVASPLGTRVTVVEQGQASYFYVASSLDAAVAASFSPRSVSIRRLKAD
ASGFAPGFVALSVLPKHLVSYSIEYVHSFHTGAFVYFLTVQPASVTDDPSALHTRLARLSATEPELGDYRELVLDCRF
APKRRRRGAPEGGQPYVLRVAHSAPVGAQLATELSIAEGQEVLFVGVFTGKDGPGVGPNSVVCAPIDLLDTL
IDEGVERCCESPVHPGLRRGLDFFQSPSFCPNPPGLEALSPNTSCRHFPLLVS SFSRVDLFNGLLGPVQVTALYVTR



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LDNVTVAHMGTM DGRILQVELVRS LN YLLYVS NFSLGDSGQPVQRDVSRLGDHLLFASGDQVFQVPIQGP GCR
HFLTCGRCLRAWHFMGCGWCGNMCGQQKECPGSWQQDHCPPKLTEEPVLIQVPLFGPRAGGTCLTLEGQSL
SVGTSRAVLVNGTECLLARVSEGQLLCATPPGATVASVPLSLQVGGAQVPGSWTFQYREDPVVLSISPNCGYINS
HITICGQHLSAWHLVLSFHDGLRAVESRCERQLPEQQLCRLPEYVVRDPQGWVAGNLSARGDGAAGFTLPGR
FLPPPHPPSANLVPLKPEEHAIFEYIGLGAVADCVGINVTVGGESCQHEFRGDMVVCPLPPSLQLGQDGAPLQV
CVDGECHILGRVVRPGPDGVPQSTLLGILLPLLLLVAALATALVFSYWWRRKQLVLPPNLNDLASLDQTAGATPLPI
LYSGSDYRSGLALPAIDGLDSTTCVHGASFSDSEDESCVPLLKESIQLRDLDSALLAEVKDVLIPHERVVTHSDRVIG
KGHFGVVYHGEYIDQAQNRIQCAIKSLSRITEMQQVEAFLREGLLMRGLNHPNVLALIGIMLPPEGLPHVLLPYM
CHGDLLQFIRSPQRNPTVKDLISFGLQVARGMEYLAEQKFVHRDLAARNCMLEDESFTVKVADFG LARDILDREYY
SVQQHRHARLPVKWMALES LQTYRFTTKSDVWSFGVLLWELLTRGAPPYRHIDPFDLTHFLAQGRRLPQPEYCP
DSLYQVMQQCWEADPAVRPTFRVLVGEVEQIVSALLGDHYVQLPATYMN LGPST SHEMNVRPEQPQFSPMPG
NVRPRPLSEPPRPTENLYFQGS DYKDDDDKG HHHHHHHHHHH

Number of amino acids: 1318

Molecular weight: 143722.30

Theoretical pI: 5.98

Extinction coefficients:

Extinction coefficients are in units of $M^{-1} cm^{-1}$, at 280 nm measured in water.

Ext. coefficient 124785

Abs 0.1% (=1 g/l) 0.868, assuming all pairs of Cys residues form cystines

>RON_Δ155-TEV-FLAG-10His_pTT5

MELLPLPQSFLLLLLPAKPAAGEDWQCPRTPYAASRDFDVKYVVP SFSAGGLVQAMVTEGDRNESAVFVAIR
NRLHVLGPDLKSVQSLATGPAGDPGCQTCAACGPGPHGPPGDTDTKVLVLDPALPALVSCGSSLQGRCLHDLE
PQGTAVHLAAPACLFSAHHNRDDCPDCVASPLGTRVTVVEQGQASYFYVASSLDAVAASFSPRSVSIRRLKAD
ASGFAPGFVALSVLPKHLVSYSIEYVHSFHTGAFVYFLTVPASVTDDPSALHTRLARLSATEPELGDYRELVLDCRF
APKRRRRGAPEGGQPYVLRVAHSAPVGAQLATELSIAEGQEVLFVGVFTGKDGGPGVGPNSVVCAPIDLLDTL
IDEGVERCCESPVHPGLRRGLDFQSPSFCPNPPGLEALSPNTSCRHFPLLSSSSFSRVDLFNGLLGPVQVTALYVTR
LDNVTVAHMGTM DGRILQVELVRS LN YLLYVS NFSLGDSGQPVQRDVSRLGDHLLFASGDQVFQVPIQGP GCR
HFLTCGRCLRAWHFMGCGWCGNMCGQQKECPGSWQQDHCPPKLTEEPVLIQVPLFGPRAGGTCLTLEGQSL
SVGTSRAVLVNGTECLLARVSEGQLLCATPPGATVASVPLSLQVGGAQVPGSWTFQYREDPVVLSISPNCGYINS
HITICGQHLSAWHLVLSFHDGLRAVESRCERQLPEQQLCRLPEYVVRDPQGWVAGVCVDGECHILGRVVRPGP
DGVPQSTLLGILLPLLLLVAALATALVFSYWWRRKQLVLPPNLNDLASLDQTAGATPLPILYSGSDYRSGLALPAIDG
LDSTTCVHGASFSDSEDESCVPLLKESIQLRDLDSALLAEVKDVLIPHERVVTHSDRVIGKGHFGVVYHGEYIDQA
QNRIQCAIKSLSRITEMQQVEAFLREGLLMRGLNHPNVLALIGIMLPPEGLPHVLLPYMCHGDLLQFIRSPQRNPT
VKDLISFGLQVARGMEYLAEQKFVHRDLAARNCMLEDESFTVKVADFG LARDILDREYYSVQQHRHARLPVKWM
ALES LQTYRFTTKSDVWSFGVLLWELLTRGAPPYRHIDPFDLTHFLAQGRRLPQPEYCPDSLYQVMQQCWEADP
AVRPTFRVLVGEVEQIVSALLGDHYVQLPATYMN LGPST SHEMNVRPEQPQFSPMPGNVRRPRPLSEPPRPTEN
LYFQGS DYKDDDDKG HHHHHHHHHHH

Number of amino acids: 1226

Molecular weight: 134151.36

Theoretical pI: 6.05

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Extinction coefficients:

Extinction coefficients are in units of $M^{-1} cm^{-1}$, at 280 nm measured in water.

Ext. coefficient 123045

Abs 0.1% (=1 g/l) 0.917, assuming all pairs of Cys residues form cystines

Summary

RON-wt

0.749 mg/mL

10 x 200 μ L (1.498 mg)

Buffer: 100 mM HEPES pH 7.5, 150 mM NaCl, 0.04% DDM

Batch No. RFR34

RON- Δ 160 (mtB)

0.858 mg/mL

9 x 200 μ L (1.544 mg)

Buffer: 100 mM HEPES pH 7.5, 150 mM NaCl, 0.04% DDM

Batch No. RFR38

RON- Δ 155 (mtA)

0.963 mg/mL

8 x 200 μ L (1.541 mg)

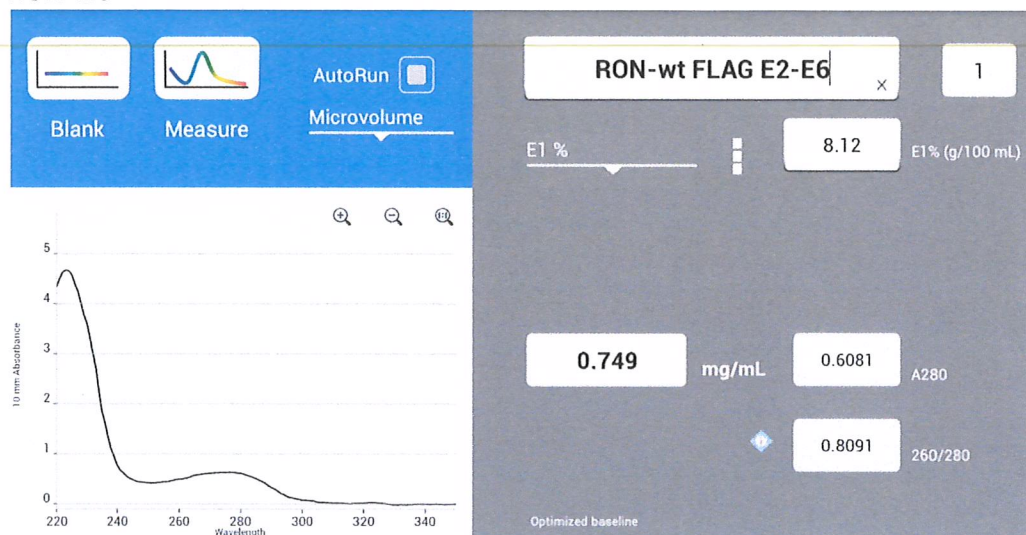
Buffer: 100 mM HEPES pH 7.5, 150 mM NaCl, 0.04% DDM

Batch No. RFR36

14 April 2020

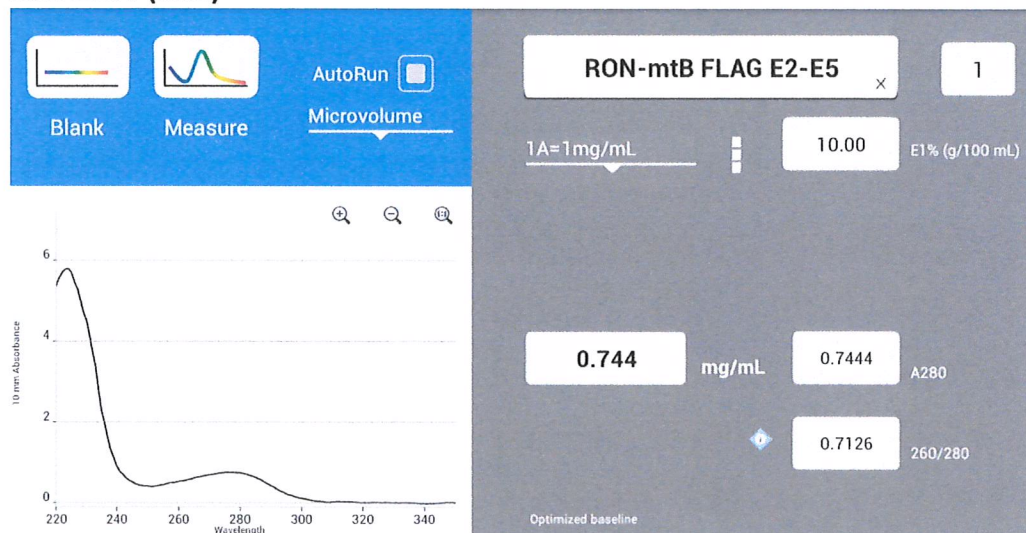
A280nm Absorbance for Protein Estimation

RON-wt



0.749 mg/mL

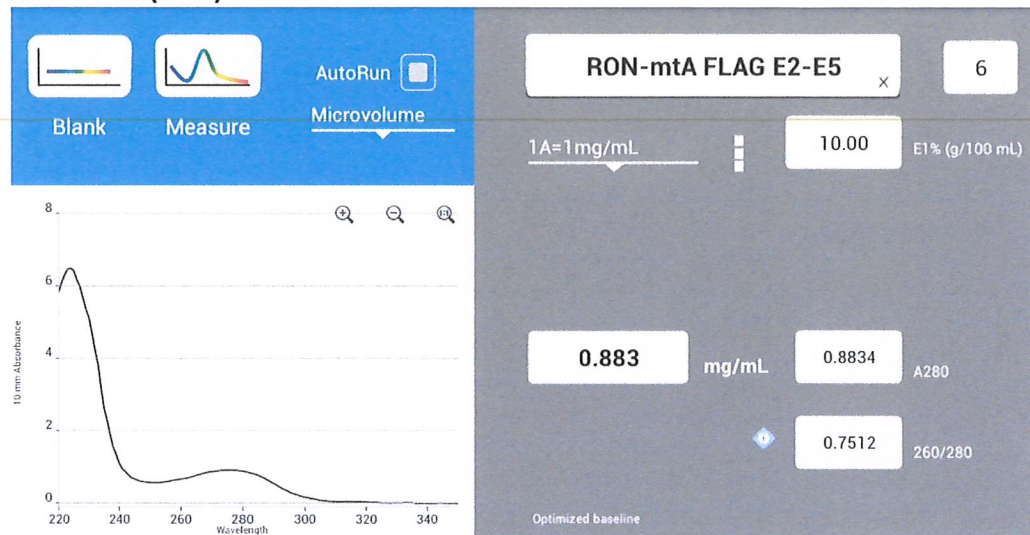
RON-Δ160 (mtB)



$0.7444/0.868 = \underline{0.858 \text{ mg/mL}}$

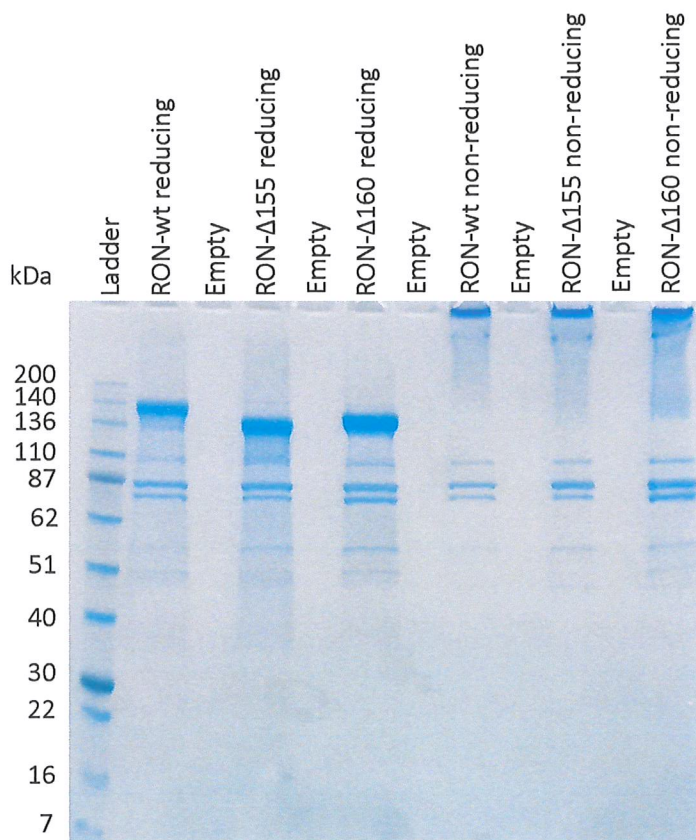
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RON-Δ155 (mtA)



$$0.8834/0.917 = 0.963 \text{ mg/mL}$$

SDS PAGE Analysis



Novex Wedgewell 10-20% Tris-glycine 12 well gel (Invitrogen, XP10202BOX)

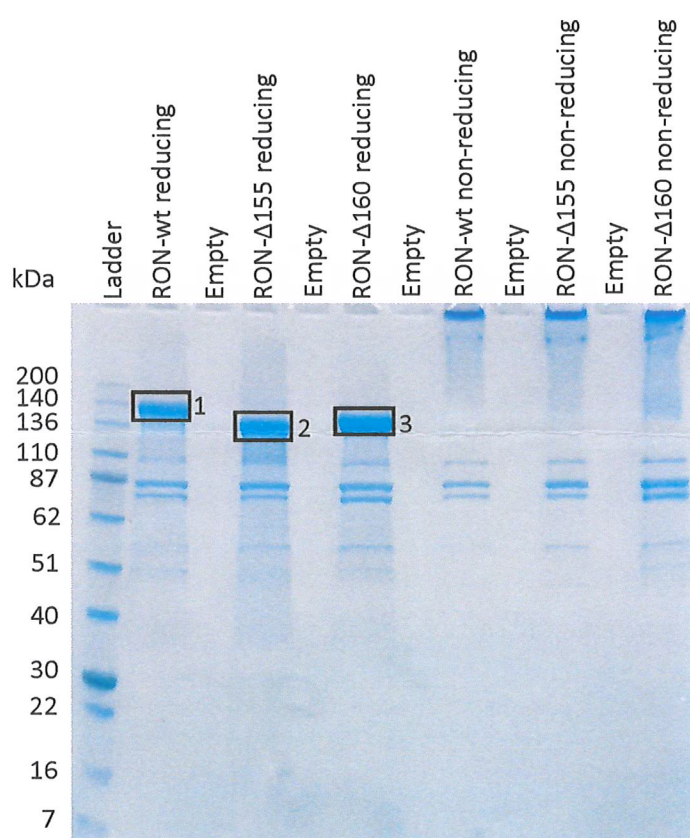
Ladder: 3 μL RunBlue TriColour pre-stained (Expedeon, NXA6050)

Protein purity >80%

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Peptide Mapping of Purified band using SCIEX x500b

The bands were reduced, alkylated and digested with trypsin and chymotrypsin. The resultant peptides were run on the Exion LC coupled to the X500B mass spectrometer, a 10 minute reversed phase gradient was used. The data was searched against Swissprot using Mascot Daemon and the sequences above were searched using the BioPharmaView software to determine coverage. Yellow highlighted sequence equates to peptides identified by mass spec.



Lane 2 – RON-wt reducing, band 1

Match to RON-wt: 64.5%

```
MELLPLPQSFLLLLPAKPAAGEDWQCPRTPYAASRDFDVKYVVPFSFAGGLVQAMVTYEGDRNESAVFVAIRNRLHV
LGPLDKSVQSLATGPGADPGCQTCAACGPGPHGPPGDDTDTKVLVLDPALPALVSCGSSSLQGRCLHDLFPQGTAVHLAAP
ACLFSAHHNRPDPCDCVASPLGTRVTVVEQGOASYFYVASSLDAVAASFSPRSVSIRRLKADAGGFAPGFVALSVLPK
HLVSYSTIEYVHSFHTGAFYFVFTVQPAVSATDDPSALHTRLARLSATEPELGDYRELVLDCRFAPKRRRRGAPEGGQPYFV
LRVAHSAPVGAQELATELSIAEQEVLFGVFTVGKGGPGVGPNSVVCAPFIDLLDTLIDEGVERCCESPVHPGLRGLDF
FQSPSPFCNPPLGLEALSPNTSCRHFPVLLVSSSFVRVDLFNGLLGPVQVTALYVTRLDNVTVAHMGTMMDGRILQVELVRS
NYLLYVSNFSLGDSGQPVQDVSRLGDLHLFASGDQVFOVPIQGPGRHFLTCGRCLRAWHFMCGCWCNMCGQKECPG
SWQDHCPCPKLTFEHPHSGPLRGSTRLTLCGSNFYLPVSGLVPEGTHQVTVGQSPCRPLPKDSSKLRPVPRKDFVEEFEC
ELEPLGTQAVGPTNVSLTVTNMPPGKHFRVDGTSVLRGFSFMEPVLIQVPLFGPRAGGTCLTLEGQSLVGTGRAVLN
GTECLLARVSEGLLQCATPPGATVASVPLSLQVGGAGVPGSWTFQYREDPVVLSISFNCGYINSHITICGQHLTSAWHLV
LSFHDGLRAVESRCERQLPEQQLCRLPEYVVRDPQGWVAGNLSARGDGAAGFTLPGFRFLPPPHPPSANLVPLKPEEHA
KFEYIGLGAVADCVGINVTVGESQCEHFRGDMVVCPLPPLSLQLGQDGAFLQVCVDGECHEILGRVVRPGPDGVPGSTLLG
ILLPLLLVAALATALVFSYWWRRRLQVLPPNLDLASLDQTAGATPLPILYSGSDYRSGALPAIDGLDSTTCVHGASF
SDSEDESCVPLLRKESIQLRDLDSALLAEVKDVLIPHERVVTHSDRVIGKGFVGVYHGEYIDQANRIQCAIKSLSRIT
EMQQVEAFLREGLLMRGLNHNPNVLAALIGIMLPEGLPHVLLPYMCHGDLLOFIRSPQRNPTVKDLISFGLQVARGMEYLA
EQKFVHRDLAARNCMLEDSFTVKVADFGDLARDILDREYYSVQQRHARLPVKWMALESLOTYRFTTKSDVVSFGVLLWEL
LTRGAPPYRHIDPFDLTHFLAQGRRLPQPEPCPSLSYQVMQCCWEADPAVRPTFRVLVGEVEQIVSALLGDHYVQLPATY
MNLGPSTSEHMNVRRPQPFSEMPGNVRRRPLSEPPRPTEPLYFQGSYKDDDDKGHHHHHHHHHH
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Lane 4 – RON-Δ155 reducing, band 2

Match to RON-Δ155: 65.9%

```
MELLPLPQSFLLLLLLPAKPAAGEDWQCPRTPYAASRDFDVKYVVPFSAGGLVQAMVTYEGDRNESAVFVAIRNRLHV
LGPDLKSVQSLATGPAGDPGCQTCAACGPGPHGPPGDTDTKVLVLDPALPALVSCGSSLQGRCLHDLPEQGTAVHLAAP
ACLFSAHNNRPDDCPDCVASPLGTRVTVVEQQQASYFYVASSLDAVAASFSRPSVSIIRLKADASGFAPGFVALSVLPK
HLVSYSEYVHSFHTGAFVYFLTVQPASVTDPSALHTRLARLSATEPELGDYRELVLDCRFAPKRRRRGAPEGGQPYPV
LRVAHSAPVGAQLATELSIAEGQEVLFVGVFTGKDGPGVGPNSVVCAPFIDLLDTLIDEGVERCCESPVHPGLRRGLDF
FQSPSFCPNPPGLEALSPNTSCRHFPLLVSSFSRVDLFNGLLGPVQVTALYVTRLDNVTVAHGMTMDGRILQVELVRSI
NYLLYVSNFSLGDSGQPVQORDVSRGLGDHLLFASGDQVQVPIQGGPCRHLTCGRCLRAWHFMGCGWCGNMGCGQKECPG
SWQDHCPPKLTTEEPVLIQVPLFGPRAGGTCLTLEGQSLSVGTSRAVLVNGTECLLARVSEGQLLCATPPGATVASVPL
SLQVGGAQVPGSWTFQYREDPVVLSISPNCGYINSHITICGQHLTSAWHLVLSFHDGLRAVESRCERQLPEQQLCRLPEY
VVRDPQGWVAGVCDGGECHILGRVVRPGDPGVQSTLLGILLPLLLLVAALATALVFSYWWRRKQLVLPNLDLASLDQ
TAGATPLPILYSGSDYRSGALPAIDGLDSTTCVHGASFSDESECVPLLRKESIQLRDLDSALLAEVKDVLIPHERVV
THSDRVIGKGHFGVYHGEYIDQQRNIQCAIKSLSRITEMQOQVEAFLREGLLMRGLNHPNVLALIGIMLPPEGLPHVLL
PYMCHGDLQLFIRSPQRNPTVKDLISFGLQVARGMEYLAQKFEVHRDLAARNCMLESTFKVADFGLDARDILDREYYSV
QQHRHARLPVKKMALESQTYRFTTKSDVVSFVLLWELLTRGAPPYRHIDPFDLTHFLAQGRRLPQPEYCPDSLYQVMQ
QCWEADPAVRPTFRVLVGEVEQIVSALLGDHYVQLPATYMNLPSTSHENNVREPEQPQFSPMPGNVRRPRLSEPPRTE
NLYFQGSYKDDDDKGGHHHHHHHHH
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Lane 6 – RON-Δ160 reducing, band 3

Match to RON-Δ160: 67.7%

```
MELLPLPQSFLLLLLLPAKPAAGEDWQCPRTPYAASRDFDVKYVVPFSAGGLVQAMVTYEGDRNESAVFVAIRNRLHV
LGPDLKSVQSLATGPAGDPGCQTCAACGPGPHGPPGDTDTKVLVLDPALPALVSCGSSLQGRCLHDLPEQGTAVHLAAP
ACLFSAHNNRPDDCPDCVASPLGTRVTVVEQQQASYFYVASSLDAVAASFSRPSVSIIRLKADASGFAPGFVALSVLPK
HLVSYSEYVHSFHTGAFVYFLTVQPASVTDPSALHTRLARLSATEPELGDYRELVLDCRFAPKRRRRGAPEGGQPYPV
LRVAHSAPVGAQLATELSIAEGQEVLFVGVFTGKDGPGVGPNSVVCAPFIDLLDTLIDEGVERCCESPVHPGLRRGLDF
FQSPSFCPNPPGLEALSPNTSCRHFPLLVSSFSRVDLFNGLLGPVQVTALYVTRLDNVTVAHGMTMDGRILQVELVRSI
NYLLYVSNFSLGDSGQPVQORDVSRGLGDHLLFASGDQVQVPIQGGPCRHLTCGRCLRAWHFMGCGWCGNMGCGQKECPG
SWQDHCPPKLTTEEPVLIQVPLFGPRAGGTCLTLEGQSLSVGTSRAVLVNGTECLLARVSEGQLLCATPPGATVASVPL
SLQVGGAQVPGSWTFQYREDPVVLSISPNCGYINSHITICGQHLTSAWHLVLSFHDGLRAVESRCERQLPEQQLCRLPEY
VVRDPQGWVAGNLSARGDGAAGFTLPGRFLPPPHPPSANLVPLKPEEHAIKFEYIGLGAADCVGINVTVGGESQHEF
RGDMVVCPLPPSLQGLQDGAFLQVCDGGECHILGRVVRPGDPGVQSTLLGILLPLLLLVAALATALVFSYWWRRKQLV
LPNLDLASLDQTAGATPLPILYSGSDYRSGALPAIDGLDSTTCVHGASFSDESECVPLLRKESIQLRDLDSALLAE
VKDVLIPHERVTHSDRVIGKGHFGVYHGEYIDQQRNIQCAIKSLSRITEMQOQVEAFLREGLLMRGLNHPNVLALIGI
MLPPEGLPHVLLPYMCHGDLQLFIRSPQRNPTVKDLISFGLQVARGMEYLAQKFEVHRDLAARNCMLESTFKVADFG
ARDILDREYYSVQQHRHARLPVKKMALESQTYRFTTKSDVVSFVLLWELLTRGAPPYRHIDPFDLTHFLAQGRRLPQ
EYCPDSLYQVMQQCWEADPAVRPTFRVLVGEVEQIVSALLGDHYVQLPATYMNLPSTSHENNVREPEQPQFSPMPGNVRR
PRLSEPPRPTENLYFQGSYKDDDDKGGHHHHHHHHH
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