# Big NERP-2 & TLQP-62

## Big NERP-2, TLQP-62, and proteolytic fragments of VGF protein C-terminus

Neuroendocrine regulatory peptide (NERP)-1 and NERP-2 are biologically active peptides recently discovered by peptidomic analysis. NERPs are processed out from the 594-residue VGF protein which contains many prohormone convertase cleavage motifs. VGF-deficient mice exhibit a hypermetabolic and infertile phenotype, for which VGF protein-derived peptides including NERPs are presumably responsible. To provide a solid basis for elucidating physiological roles of NERPs, we investigated rat VGF protein processing by chromatographic and mass spectrometric analysis, and immunoblotting, using antibodies against NERPs and the VGF protein C-terminus (VGF-C). Cellular and tissue distribution of immunoreactive (ir) NERPs were also analyzed in the rat. Both ir-NERP-1 and ir-NERP-2, which occur abundantly in the CNS and pituitary, moderately in the gastrointestinal (GI) tract, were mainly localized in neuronal structures. Major endogenous forms of ir-NERPs in the brain and GI tract were identified as NERP-1, NERP-2, and big NERP-2 (NERP-1+NERP-2), with NERP-1 and big NERP-2 being predominant. Regarding ir-VGF-C peptides, VGF[588-617], VGF[556-617], and VGF[509-617] were found to be major forms. Immunoblotting with the NERP-2 and VGF-C antibodies revealed processing intermediates of 10-37 kDa. Taken together, we deduce that VGF protein is primarily cleaved at 10 sites through the processing pathway common to the brain and GI tract.

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**BIG NERP-2 (Human, Rat) Sequence Comparison**

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Rat                                LEGSFL GGSEAGERLL  300
Human                            RPESALL GGSEAGERLL  297

Rat   301  QQGLAQVEAG RRQAEATRQA AAQEERLADL  330
Human 298  QQGLAQVEAG RRQAEATRQA AAQEERLADL  327

Rat   331  ASDLLLQYLL QGGARQRDLG
Human 328  ASDLLLQYLL QGGARQRGLG
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**BIG NERP-2 (Human) Sequence**

RPESALL GGSEAGERLL QQGLAQVEAG RRQAEATRQA AAQEERLADL ASDLLLQYLL QGGARQRGLG

**BIG NERP-2 (Rat) Sequence**

LEGSFL GGSEAGERLL QQGLAQVEAG RRQAEATRQA AAQEERLADL ASDLLLQYLL QAGARQRDLG


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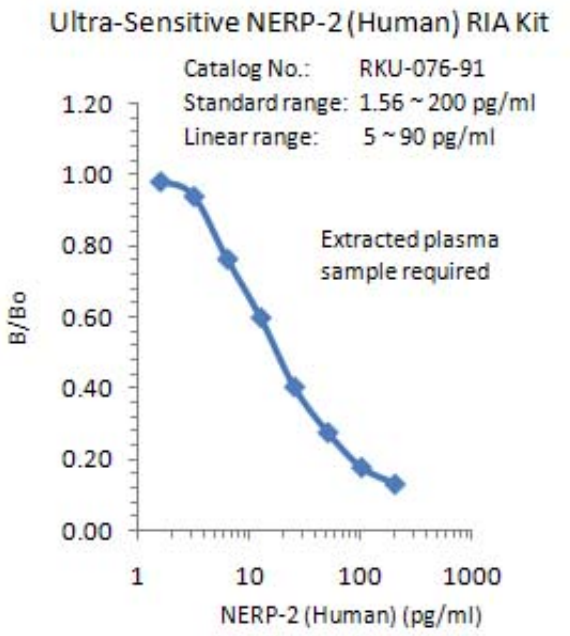
## Peptidomic identification and biological validation of neuroendocrine regulatory peptide-1 and -2.

Recent advances in peptidomics have enabled the identification of previously uncharacterized peptides. However, sequence information alone does not allow us to identify candidates for bioactive peptides. To increase an opportunity to discover bioactive peptides, we have focused on carboxy-terminal amidation, a post-translational modification shared by many bioactive peptides. We analyzed peptides secreted from human medullary thyroid carcinoma TT cells that produces amidated peptides, and identified two novel amidated peptides, designated neuroendocrine regulatory peptide (NERP) -1 and NERP-2. NERPs are derived from distinct regions of the neurosecretory protein that was originally identified as a product of a nerve growth factor-responsive gene in PC12 cells. Mass spectrometric analysis of the immunoprecipitate using specific antibodies as well as RP-HPLC coupled with radioimmunoassay analysis of brain extract demonstrated the endogenous presence of NERP-1 and NERP-2 in the rat. NERPs are abundant in the paraventricular and supraoptic nuclei of the rat hypothalamus and colocalized frequently with vasopressin, but rarely with oxytocin. NERPs dose-dependently suppressed vasopressin release induced by icv injection of hypertonic NaCl or angiotensin II in vivo. NERPs also suppressed basal and angiotensin II-induced vasopressin secretion from hypothalamic explants in vitro. Bioactivity of NERPs required carboxy-terminal amidation. Anti-NERPs IgGs canceled plasma vasopressin reduction in response to water loading, indicating that NERPs could be potent endogenous suppressors of vasopressin release. These findings suggest that NERPs are novel modulators in body fluid homeostasis.

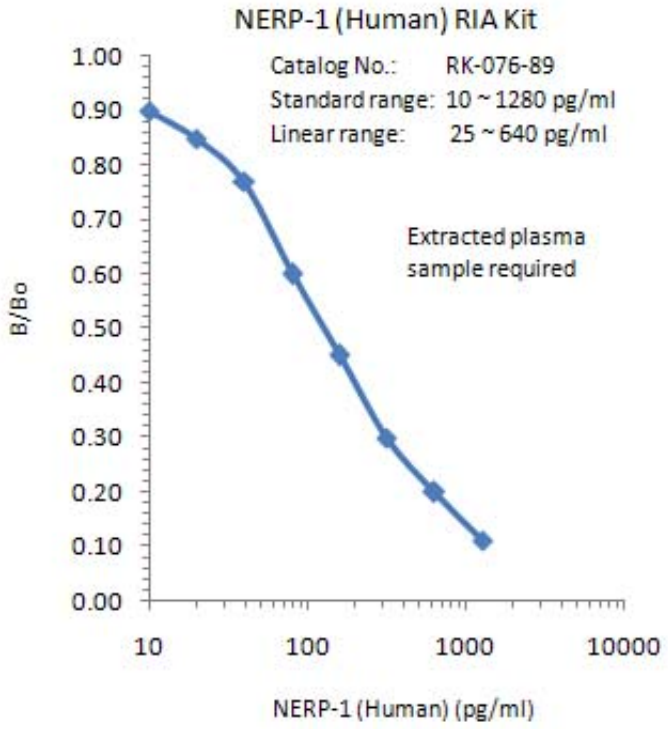
*Yamaguchi et al. J Biol Chem. 2007 Sep 7;282(36):26354-60. Epub 2007 Jul 3.*

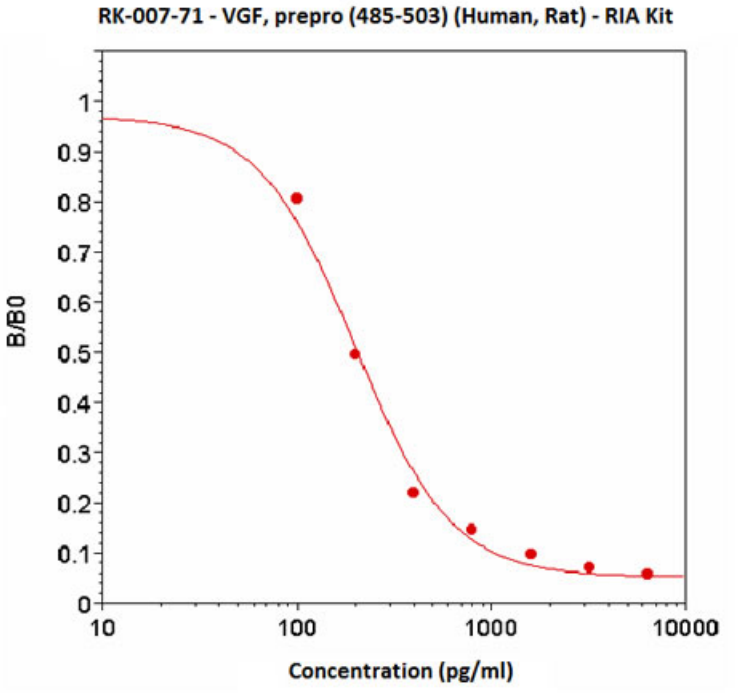



Specificity of Human NERP-2 RIA

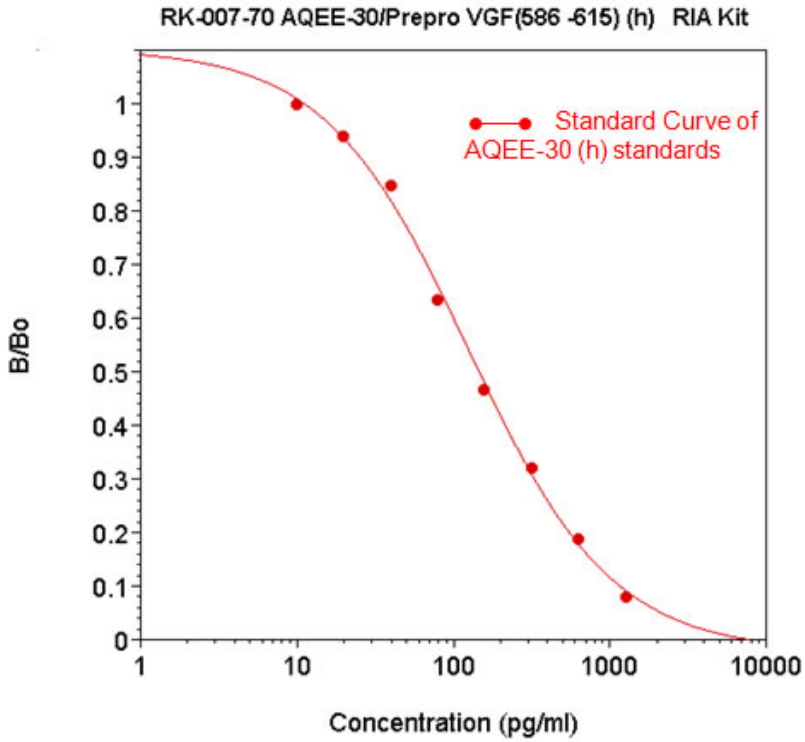
| Peptide Name | % Cross-reactivity |
|---|---|
| Human NERP-2 | 100% |
| Rat NERP-2 | 100% |
| Human NERP-1 | 0 |
| Rat NERP-1 | 0 |

All listed peptides at 2560000 pg/ml indicated crossreactivity in RIA system

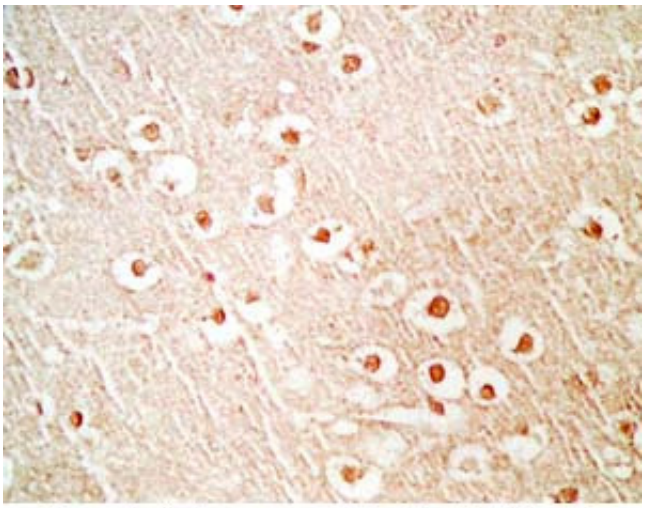



**Range: 10-1280pg/ml** **IC50: 19.80pg/ml** **Linear Range: 200-800pg/ml**



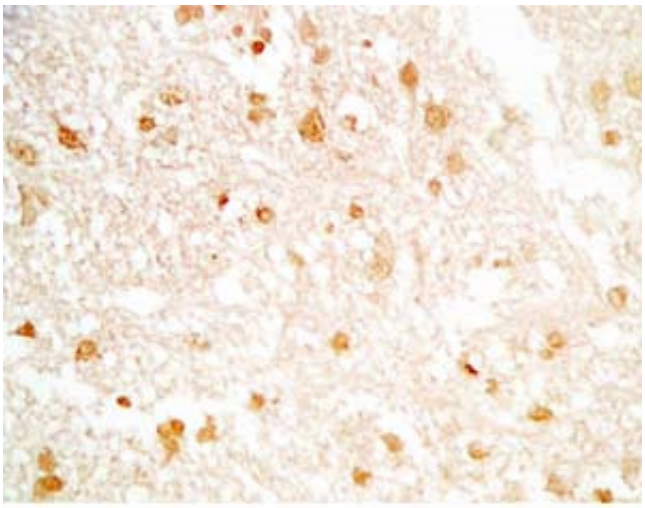

**Range: 10-1280 pg/ml** **IC50 : 125 pg/ml** **Linear Range: 45 pg/ml ~ 640 pg/ml**



Mouse brain tissue was stained by Rabbit Anti-NERP-1 (Human) Antibody (catalog No.: H-076-89)

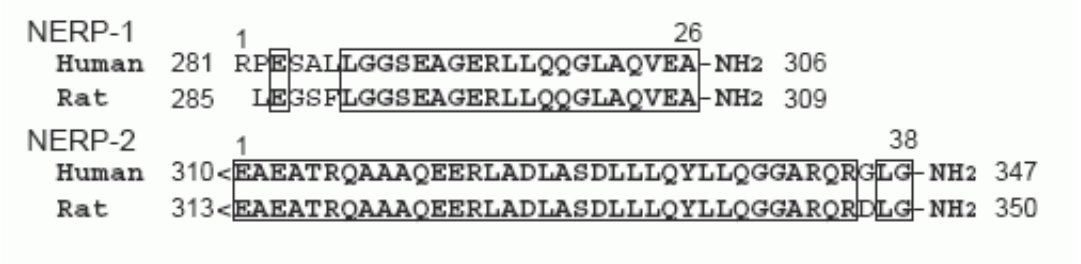
| Tissue Sample | Rat/Mouse Brain Tissue |
|---|---|
| Fixative | 10% formalin |
| Embedding | Paraffin |
| Negative Control | No primary antibody (pre-immuno serum) |
| Pretreatment | Intact |
| Blocking | 2% Normal Goat Serum |
| Primary Antibody | Rabbit Anti-NERP-1 (Human) Antibody (Catalog No.:H-076-89) |
| Optimal Dilution | 1:500 |
| Secondary Antibody | Goat Anti-Rabbit IgG, Biotinylated (1:400), 30 min |
| Amplification | ABC (Vector) (1:400, 30 min) |
| Detection System | HRP |
| Substrate | DAB (Sigma), 3 min |
| Counterstained | Hematoxylin, 30 sec |

| Tissue Sample | Rat/Mouse Brain Tissue |
|---|---|
| Fixative | 10% formalin |
| Embedding | Paraffin |
| Negative Control | No primary antibody (pre-immuno serum) |
| Pretreatment | Intact |
| Blocking | 2% Normal Goat Serum |
| Primary Antibody | Rabbit Anti-NERP-2 (Human) Antibody (Catalog No.:H-076-91) |
| Optimal Dilution | 1:500 |
| Secondary Antibody | Goat anti-Rabbit IgG, Biotinylated (1:400, 30 min) |
| Amplification | ABC (Vector) (1:400, 30 min) |
| Detection System | HRP |
| Substrate | DAB (Sigma), 3 min |
| Counterstained | Hematoxylin, 30 sec |

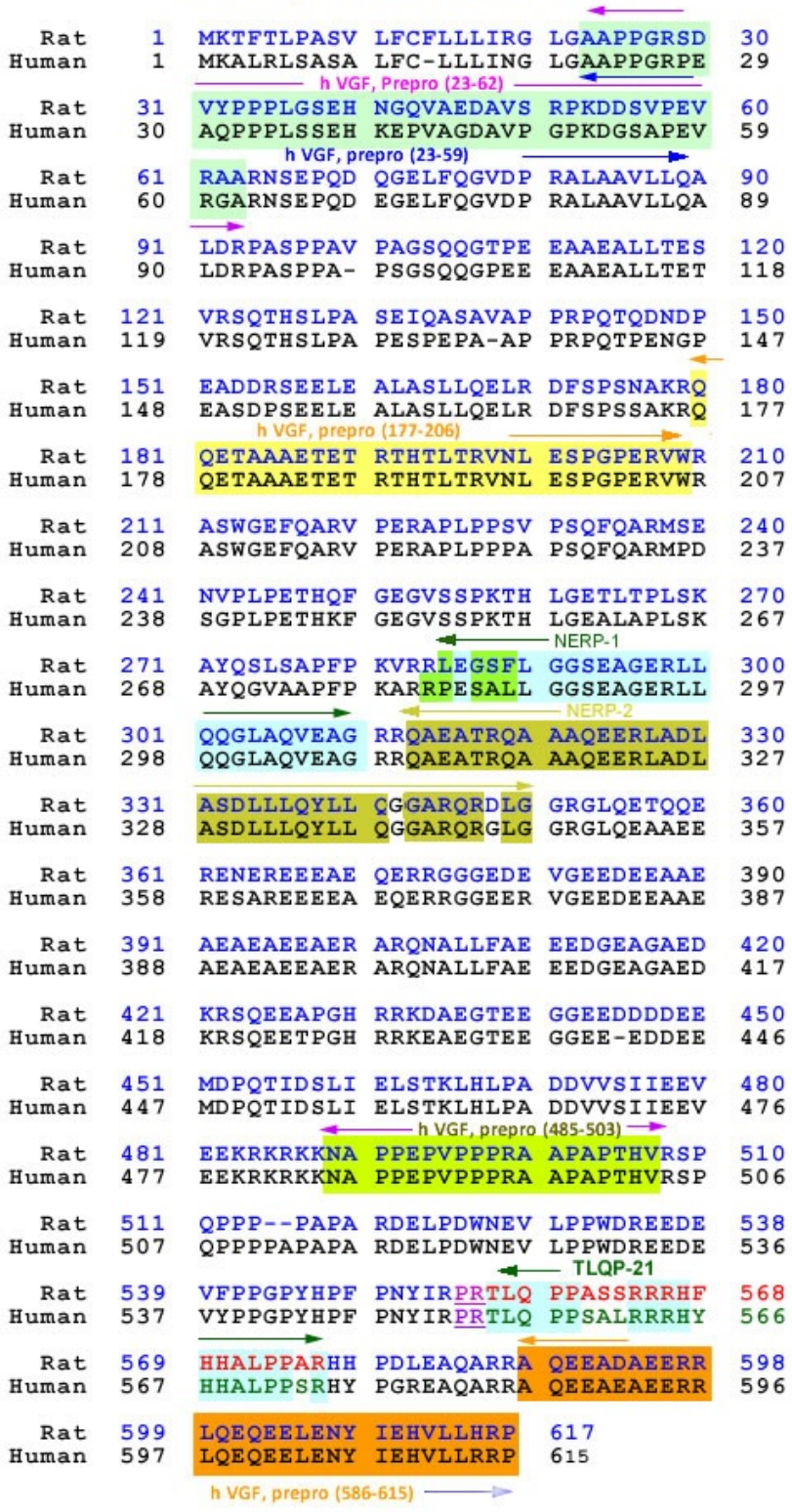


Rat brain tissue was stained by Rabbit Anti-NERP-2 (Human) Antibody (catalog No.: H-076-91)

## Amino acid Sequence of Human, Rat NERP-1 and NERP-2



## Blast sequences of rat and human VGF




| | | | |
|---|---|---|---|
| Rat | 1 | MKTFTLPASV LFCFLLLIRG LGAAPPGRSD | 30 |
| Human | 1 | MKALRLSASA LFC-LLLING LGAAPPGRPE | 29 |
| Rat | 31 | VYPPPLGSEH NGQVAEDAVS RPKDDSVPEV | 60 |
| Human | 30 | AQPPPLSSEH KEPVAGDAVP GPKDGSAPEV | 59 |
| Rat | 61 | RAARNSEPQD QGELFQGVDP RALAAVLLQA | 90 |
| Human | 60 | RGARNSEPQD EGELFQGVDP RALAAVLLQA | 89 |
| Rat | 91 | LDRPASPPAV PAGSQQGTPE EAAEALLTES | 120 |
| Human | 90 | LDRPASPPA- PSGSQQGPEE EAAEALLTET | 118 |
| Rat | 121 | VRSQTHSLPA SEIQASAVAP PRPQTQDNDP | 150 |
| Human | 119 | VRSQTHSLPA PESPEPA-AP PRPQTPENGP | 147 |
| Rat | 151 | EADDRSEELE ALASLLQELR DFSPSNAKRQ | 180 |
| Human | 148 | EASDPSEELE ALASLLQELR DFSPSSAKRQ | 177 |
| Rat | 181 | QETAAAETET RTHTLTRVNL ESPGPERVWR | 210 |
| Human | 178 | QETAAAETET RTHTLTRVNL ESPGPERVWR | 207 |
| Rat | 211 | ASWGEFQARV PERAPLPPSV PSQFQARMSE | 240 |
| Human | 208 | ASWGEFQARV PERAPLPPPA PSQFQARMPD | 237 |
| Rat | 241 | NVPLPETHQF GEGVSSPKTH LGETLTPLSK | 270 |
| Human | 238 | SGPLPETHKF GEGVSSPKTH LGEALAPLSK | 267 |
| Rat | 271 | AYQSLSAPFP KVRRLEGSFL GGSEAGERLL | 300 |
| Human | 268 | AYQGVAAPFP KARRPESALL GGSEAGERLL | 297 |
| Rat | 301 | QQGLAQVEAG RRQAEATRQA AAQEERLADL | 330 |
| Human | 298 | QQGLAQVEAG RRQAEATRQA AAQEERLADL | 327 |
| Rat | 331 | ASDLLLQYLL QGGARQRDLG GRGLQETQQE | 360 |
| Human | 328 | ASDLLLQYLL QGGARQRGLG GRGLQEAAEE | 357 |
| Rat | 361 | RENEREEEAE QERRGGGEDE VGEEDEEAAE | 390 |
| Human | 358 | RESAREEEEA EQERRGGEER VGEEDEEAAE | 387 |
| Rat | 391 | AEAEAEEAER ARQNALLFAE EEDGEAGAED | 420 |
| Human | 388 | AEAEAEEAER ARQNALLFAE EEDGEAGAED | 417 |
| Rat | 421 | KRSQEEAPGH RRKDAEGTEE GGEEDDDDEE | 450 |
| Human | 418 | KRSQEETPGH RRKEAEGTEE GGEE-EDDEE | 446 |
| Rat | 451 | MDPQTIDSLI ELSTKLHLPA DDVVSIIEEV | 480 |
| Human | 447 | MDPQTIDSLI ELSTKLHLPA DDVVSIIEEV | 476 |
| Rat | 481 | EEKRKRKKNA PPEPVPPPRA APAPTHVRSP | 510 |
| Human | 477 | EEKRKRKKNA PPEPVPPPRA APAPTHVRSP | 506 |
| Rat | 511 | QPPP--PAPA RDELPDWNEV LPPWDREEDE | 538 |
| Human | 507 | QPPPPAPAPA RDELPDWNEV LPPWDREEDE | 536 |
| Rat | 539 | VFPPGPYHPF PNYIRPRTLQ PPASSRRRHF | 568 |
| Human | 537 | VYPPGPYHPF PNYIRPRTLQ PPSALRRRHY | 566 |
| Rat | 569 | HHALPPARHH PDLEAQARRA QEEADAEERR | 598 |
| Human | 567 | HHALPPSRHY PGREAQARRA QEEAEAEERR | 596 |
| Rat | 599 | LQEQEELENY IEHVLLHRP | 617 |
| Human | 597 | LQEQEELENY IEHVLLRRP | 615 |

*Rat TLQP-21 peptide was identified by*
*Bartolomucci A., et al. PNAS, 2006, 103, 14584-14589*
*Nov. 03, 2006, Phoenix Pharmaceuticals, Inc.*
*July. 09, 2007, Phoenix Pharmaceuticals, Inc.*

**TLQP-62 (Human, Rat) Sequence Comparison**


**TLQP-62 (Human) Sequence**

TLQ PPSALRRRHY HHALPPSRHY PGREAQARRA QEEEAEAEERR LQEQEELENY IEHVLLRRP

**TLQP-62 (Rat) Sequence**

TLQ PPASSRRRHF HHALPPARHH PDLEAQARRA QEEEADAEERR LQEQEELENY IEHVLLHRP

| Catalog No. | Name | Size |
|---|---|---|
| 007-65 | VGF, prepro (23-62) (Human, Rat) | 100 µg |
| 007-66 | VGF, prepro (23-59) (Human, Rat) | 100 µg |
| 007-67 | VGF, prepro (177-206) (Human) | 100 µg |
| 007-68 | VGF, prepro (177-206) [pGlu1] (Human) | 100 µg |
| 007-70 | AQEE-30 / VGF, prepro (586-615) (Human) | 100 µg |
| 007-73 | AQEE-19 / VGF, prepro (597-615) (Human) | 200 µg |
| 076-89 | NERP-1 / VGF, prepro (281-306) (Human) | 100 µg |
| 076-90 | NERP-1 (Rat) | 100 µg |
| 076-91 | NERP-2 (Human) | 100 µg |
| 076-92 | NERP-2 (Rat) | 100 µg |
| B-076-89 | NERP-1 / VGF, prepro (281-306) (Human) - Biotin Labeled | 20 µg |
| B-076-90 | NERP-1 (Rat) - Biotin Labeled | 20 µg |
| B-076-91 | NERP-2 (Human) - Biotin Labeled | 20 µg |
| B-076-92 | NERP-2 (Rat) - Biotin Labeled | 20 µg |
| B-G-076-89 | NERP-1 / VGF, prepro (281-306) (Human) - Biotin Labeled Purified IgG | 100 µl |
| B-G-076-91 | NERP-2 (Human) - Biotin Labeled Purified IgG | 100 µl |
| B-G-076-92 | NERP-2 (Rat) - Biotin Labeled Purified IgG | 100 µl |
| FC3-G-076-89 | NERP-1 / VGF, prepro (281-306) (Human) - Cy3 Labeled Purified IgG | 100 µl |
| FC3-G-076-91 | NERP-2 (Human) - Cy3 Labeled Purified IgG | 100 µl |
| FC3-G-076-92 | NERP-2 (Rat) - Cy3 Labeled Purified IgG | 100 µl |

| Catalog No. | Name | Size |
|---|---|---|
| FC5-G-076-89 | NERP-1 / VGF, prepro (281-306) (Human) - Cy5 Labeled Purified IgG | 100 µl |
| FC5-G-076-92 | NERP-2 (Rat) - Cy5 Labeled Purified IgG | 100 µl |
| FG-076-89A | NERP-1 / VGF, prepro (281-306) (Human) - FAM Labeled | 1 nmol |
| FG-076-89B | NERP-1 / VGF, prepro (281-306) (Human) - FITC Labeled | 1 nmol |
| FG-076-90A | NERP-1 (Rat) - FAM Labeled | 1 nmol |
| FG-076-90B | NERP-1 (Rat) - FITC Labeled | 1 nmol |
| FG-076-91A | NERP-2 (Human) - FAM Labeled | 1 nmol |
| FG-076-91B | NERP-2 (Human) - FITC Labeled | 1 nmol |
| FG-076-92A | NERP-2 (Rat) - FAM Labeled | 1 nmol |
| FG-076-92B | NERP-2 (Rat) - FITC Labeled | 1 nmol |
| FG-G-076-89A | NERP-1 / VGF, prepro (281-306) (Human) - FAM Labeled Purified IgG | 100 µl |
| FG-G-076-89B | NERP-1 / VGF, prepro (281-306) (Human) - FITC Labeled Purified IgG | 100 µl |
| FG-G-076-91A | NERP-2 (Human) - FAM Labeled Purified IgG | 100 µl |
| FG-G-076-91B | NERP-2 (Human) - FITC Labeled Purified IgG | 100 µl |
| FG-G-076-92A | NERP-2 (Rat) - FAM Labeled Purified IgG | 100 µl |
| FG-G-076-92B | NERP-2 (Rat) - FITC Labeled Purified IgG | 100 µl |
| FR-G-076-91 | NERP-2 (Human) - Rhodamine Labeled Purified IgG | 100 µl |
| G-076-89 | NERP-1 / VGF, prepro (281-306) (Human) - Purified IgG Antibody | 100 µg |
| G-076-91 | NERP-2 (Human) - Purified IgG Antibody | 100 µg |
| G-076-92 | NERP-2 (Rat) - Purified IgG | 100 µg |
| H-076-89 | NERP-1 / VGF, prepro (281-306) (Human) - Antibody for Immunohistochemistry | 100 µl |
| H-076-91 | NERP-2 (Human) - Antibody for Immunohistochemistry | 100 µl |
| H-076-92 | NERP-2 (Rat) - Antibody for Immunohistochemistry | 100 µl |
| RK-007-70 | AQEE-30 / VGF, prepro (586-615) (Human) - RIA Kit | 1 kit |
| RK-076-89 | NERP-1 / VGF, prepro (281-306) (Human) - RIA Kit | 1 kit |
| RKU-076-91 | NERP-2 (Human) - Ultra-Sensitive RIA Kit | 1 kit |
| T-076-91 | NERP-2 (Human) - I-125 Labeled | 10 µCi |
| T-076-92 | NERP-2 (Rat) - I-125 Labeled | 10 µCi |