Norrin Mutant [L61N/A63S] Vector

Catalogue number: 152593
Sub-type: pHLIgK-STR-8H-SUMO-1D4
Images:

Contributor

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Institute: University of Oxford
Images:

Tool details

***FOR RESEARCH USE ONLY**

Name: Norrin Mutant [L61N/A63S] Vector

Alternate name:

Class:
Conjugate:
Description: Plasmid is available in 10 ug aliquots. Full sequence available on request. Norrin recombinant protein expression and purification protocol Norrin was expressed in HEK293T cells in the presence of 4 mM valproic acid, a histone deacetylase inhibitor (Backliwal et al., 2008), used to increase the expression level of secreted protein. The detailed expression protocol was described in our publication (Chang et al., 2015). Norrin conditioned media (500 ml) were dialyzed against 5L of PBS buffer plus 0.4 M NaCl for 24 hrs. The dialyzed media were adjusted to 20 mM Tris, pH 8.0 and 2.5 mM Imidazole, pH 7.5. Recombinant protein was further purified from the adjusted media by IMAC (TALONÄ?Â?ÄšË•Clontech), washed with 25 mM Tris, pH7.5, 0. 5 M NaCl, 0.02 M Imidazole, 10 % [w/v] Glycerol, and eluted in 25 mM Tris, pH7.5, 0.15 M NaCl, 0.5 M Imidazole. The purified sample was added CHAPS to 1% [w/v] and dialyzed against 25 mM Tris, pH 7.5, 1M NaCl, 10% [w/v] Glycerol, before treating with His-tagged HRV-3C protease to remove the SUMOtagged fusion protein. The untagged sample was further isolated by IMAC (collection of flow-through and wash with 25 mM Tris, pH7.5, 1 M NaCl, 0.01 M Imidazole, 1% [w/v] CHAPS) and purified by SEC (SEC, Superdex 200 10/300 GL High Performance, GE Healthcare Life Sciences) in 10 mM HEPES, pH 7.5, 0.7 M NaCl, 0.5% [w/v] CHAPS or 10 mM acetate buffer, pH 4.0, 0.5 M NaCl, 0.5% [w/v] CHAPS. Recombinant protein can be stored in 10mM acetate buffer, pH 4.0, 0.5 M NaCl, 0.5% [w/v]CHAPS or in 10 mM HEPES, pH 7.5, 0.7 M NaCl, 0.5% [w/v] CHAPS. Norrin (residues 25-133) L61N/A63S KTDSSFIMDSDPRRCMRHHYVDSISHPLYKCSSKMVNLSRCEGHCSQASRSEPLVSFSTVLKQPFRSSCH
Purpose:
Parental cell:
Organism:
Tissue:

Model:
Gender:
Isotype:
Reactivity:
Selectivity:
Host:
Immunogen:
Immunogen UNIPROT ID:
Sequence:
Growth properties:
Production details:
Formulation:
Recommended controls:
Bacterial resistance:
Selectable markers:
Additional notes: Norrin (Norrie Disease Protein) is a cystine-knot like growth factor that can activate Wnt signalling by binding to Frizzled and another receptor protein called Lrp5/6. This group or â€˜complexâ€™ also includes molecules called glycosaminoglycans. The L61N/A63S mutant loses signalling activity and binding ability to Frizzled 4 receptor, but retains the interactions with coâ€?receptors of low density lipoprotein related protein 5/6 (Lrp5/6) and heparan sulphate proteoglycans (HSPGs). Wnt signalling regulates multiple processes including angiogenesis, inflammation, and tumorigenesis. In humans, mutations in the gene that encodes Norrin can cause a disease in which blood vessels in the eye fail to form correctly, which can result in blindness. However, it is not clear how Norrin activates Wnt signalling.

Target details

Target: Human Norrin (Norrie Disease Protein, NDP)

Target alternate names:

Target background:

Molecular weight:

Ic50:

Applications

Application:
Application notes: Plasmid is available in 10 ug aliquots. Full sequence available on request. Norrin recombinant protein expression and purification protocol Norrin was expressed in HEK293T cells in the presence of 4 mM valproic acid, a histone deacetylase inhibitor (Backliwal et al., 2008), used to increase the expression level of secreted protein. The detailed expression protocol was described in our publication (Chang et al., 2015). Norrin conditioned media (500 ml) were dialyzed against 5L of

PBS buffer plus 0.4 M NaCl for 24 hrs. The dialyzed media were adjusted to 20 mM Tris, pH 8.0 and 2.5 mM Imidazole, pH 7.5. Recombinant protein was further purified from the adjusted media by IMAC (TALONÂ®Clontech), washed with 25 mM Tris, pH7.5, 0. 5 M NaCl, 0.02 M Imidazole, 10 % [w/v] Glycerol, and eluted in 25 mM Tris, pH7.5, 0.15 M NaCl, 0.5 M Imidazole. The purified sample was added CHAPS to 1% [w/v] and dialyzed against 25 mM Tris, pH 7.5, 1M NaCl, 10% [w/v] Glycerol, before treating with His-tagged HRV-3C protease to remove the SUMOtagged fusion protein. The untagged sample was further isolated by IMAC (collection of flow-through and wash with 25 mM Tris, pH7.5, 1 M NaCl, 0.01 M Imidazole, 1% [w/v] CHAPS) and purified by SEC (SEC, Superdex 200 10/300 GL High Performance, GE Healthcare Life Sciences) in 10 mM HEPES, pH 7.5, 0.7 M NaCl, 0.5% [w/v] CHAPS or 10 mM acetate buffer, pH 4.0, 0.5 M NaCl, 0.5% [w/v] CHAPS. Recombinant protein can be stored in 10mM acetate buffer, pH 4.0, 0.5 M NaCl, 0.5% [w/v]CHAPS or in 10 mM HEPES, pH 7.5, 0.7 M NaCl, 0.5% [w/v] CHAPS. Norrin (residues 25-133) L61N/A63S KTDSSFIMDSDPRRCMRHHYVDSISHPLYKCSSKMVNLSRCEGHCSQASRSEPLVSFSTVLKQPFRSSCH

Handling

Format:
Concentration:
Passage number:
Growth medium:
Temperature:
Atmosphere:
Volume:
Storage medium:
Storage buffer:
Storage conditions:
Shipping conditions:

Related tools

Related tools: Norrin WT Vector ; Norrin Mutant [R107E/R109E/R115L] Vector

References

References: Original hybridoma first published in Palmer et al. 1985. Clin Exp Immunol. 59(3):529-38. PMID: 3886218. ; Features of synovial membrane identified with monoclonal antibodies.