# Human Sortilin Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF3154

## DESCRIPTION

| | |
|---|---|
| **Species Reactivity** | Human |
| **Specificity** | Detects human and mouse Sortilin in direct ELISAs and Western blots. |
| **Source** | Polyclonal Goat IgG |
| **Purification** | Antigen Affinity-purified |
| **Immunogen** | Mouse myeloma cell line NS0-derived recombinant human Sortilin<br>Ser78-Asn755<br>Accession # Q99523 |
| **Endotoxin Level** | <0.10 EU per 1 µg of the antibody by the LAL method. |
| **Formulation** | Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.<br>*Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS. |

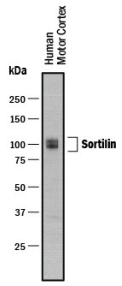
## APPLICATIONS

**Please Note:** *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

| | Recommended Concentration | Sample |
|---|---|---|
| **Western Blot** | 2 µg/mL | See Below |
| **Flow Cytometry** | 0.25 µg/$10^6$ cells | See Below |
| **Immunohistochemistry** | 5-15 µg/mL | See Below |
| **Simple Western** | 10 µg/mL | See Below |
| **Blockade of Receptor-ligand Interaction** | In a functional ELISA, 3-9 µg/mL of this antibody will block 50% of the binding of 200 ng/mL of Recombinant Human β-NGF (Catalog # 256-GF) to immobilized Recombinant Human Sortilin (Catalog # 3154-ST) coated at 4 µg/mL (100 µL/well). At 100 µg/mL, this antibody will block >90% of the binding. | |

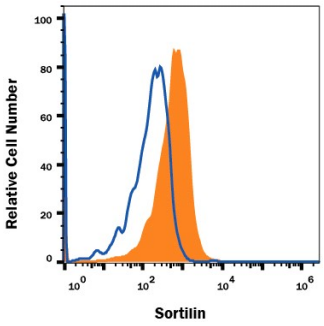
## DATA

### Western Blot



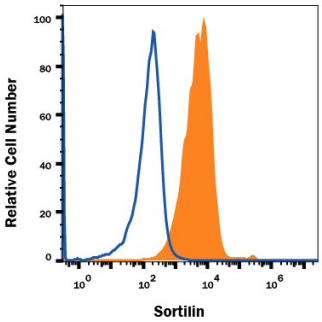

**Detection of Human Sortilin by Western Blot.** Western blot shows lysates of human motor cortex. PVDF membrane was probed with 2 µg/mL of Goat Anti-Human Sortilin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3154) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for Sortilin at approximately 95-105 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

### Flow Cytometry



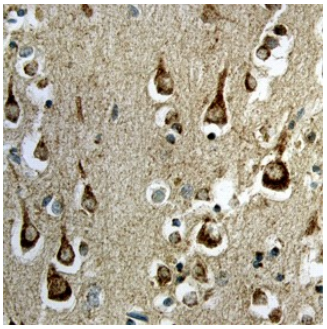

**Detection of Sortilin in Human Blood Monocytes by Flow Cytometry.** Human peripheral blood monocytes were stained with Goat Anti-Human Sortilin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3154, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108). View our protocol for Staining Membrane-associated Proteins.

### Flow Cytometry




**Detection of Sortilin in K562 Human Cell Line by Flow Cytometry.** K562 human chronic myelogenous leukemia cell line was stained with Goat Anti-Human Sortilin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3154, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108). View our protocol for Staining Membrane-associated Proteins.

### Immunohistochemistry



**Sortilin in Human Brain.** Sortilin was detected in immersion fixed paraffin-embedded sections of human brain using Goat Anti-Human Sortilin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3154) at 1.7 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.



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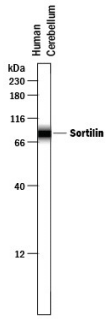
**Simple Western**




**Detection of Human Sortilin by Simple Western™.** Simple Western lane view shows lysates of human brain (cerebellum) tissue, loaded at 0.2 mg/mL. A specific band was detected for Sortilin at approximately 82 kDa (as indicated) using 10 µg/mL of Goat Anti-Human Sortilin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3154) . This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

## PREPARATION AND STORAGE

| | |
|---|---|
| **Reconstitution** | Reconstitute at 0.2 mg/mL in sterile PBS. |
| **Shipping** | The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.<br>*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C |
| **Stability & Storage** | **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**<br>● 12 months from date of receipt, -20 to -70 °C as supplied.<br>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.<br>● 6 months, -20 to -70 °C under sterile conditions after reconstitution. |

## BACKGROUND

Sortilin (neurotensin receptor 3, glycoprotein 95) is a 95 kDa Type I transmembrane monomeric glycoprotein that is one of five known members of the mammalian vacuolar protein sorting 10p domain (Vps10p-D) family of sorting receptors (1, 2). Human preprosortilin is processed by signal sequence cleavage followed by propeptide cleavage at a furin recognition site. The cationic propeptide exhibits pH-dependent high affinity binding that blocks the Sortilin ligand binding site both pre- and post-cleavage (3). The extracellular/luminal sequence comprises the Vps10p domain, including 10 conserved cysteines (10 CC) essential for ligand binding (2). The cytoplasmic domain sorting motifs confer all trafficking during synthesis, targeting to lysosomes, endocytosis and Golgi-endosome transport; as little as 10% may be found on the cell surface (4). Mature human Sortilin shares 91% aa identity with mouse and rat Sortilin and 93% aa identity with dog. During murine development, sortilin is mainly expressed in the nervous system (5) where it is a receptor for neuropeptides including neurotensin, nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) (6-9). ProNGF (or the NGF propeptide alone) binds sortilin with much higher affinity (Kd ~5-8 nM) than does mature NGF (Kd ~90 nM). The complex of sortilin, pro-NGF and the receptor $p75^{ntr}$ results in endocytosis of proNGF and induction of apoptosis (7). Similar results have been obtained with pro-BDNF and BDNF (8, 9). Sortilin is expressed in other tissues including testis, skeletal muscle and fat (1, 10). It is essential and sufficient for biogenesis of Glut4 storage vesicles necessary for insulin responsiveness in adipocytes (10). Sortilin also binds lipoprotein lipase (11), apoE (2) and RAP (1, 11). Binding is competitive, indicating that although unrelated, targets likely bind the same site.

**References:**

1. Petersen, C.M. *et al.* (1997) J. Biol. Chem. **272**:3599.
2. Westergaard, U.B. *et al.* (2004) J. Biol. Chem. **279**:50221.
3. Petersen, C.M. *et al.* (1998) EMBO J. **18**:595.
4. Nielsen, M.S. *et al.* (2001) EMBO J. **20**:2180.
5. Hermans-Borgmeyer, I. *et al.* (1999) Mol. Brain Res. **65**:216.
6. Mazella, J. *et al.* (1998) J. Biol. Chem. **273**:26273.
7. Nykjaer, A. *et al.* (2004) Nature **427**:843.
8. Teng, H.K. *et al.* (2005) J. Neurosci. **25**:5455.
9. Chen, Z-Y. *et al.* (2004) J. Neurosci. **25**:6156.
10. Shi, J. and K.V. Kandror (2005) Dev. Cell **9**:99.
11. Nielsen, M.S. *et al.* (1999) J. Biol. Chem. **274**:8832.



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