## **Notch Receptor Interaction Antibody Sampler Kit**

✓ 1 Kit  $(9 \times 20 \mu I)$ 



**Orders** 877-616-CELL (2355)

orders@cellsignal.com

**Support** 877-678-TECH (8324)

info@cellsignal.com

Web www.cellsignal.com

rev. 03/07/16

## For Research Use Only. Not For Use In Diagnostic Procedures.

| Products Included                    | Product # | Quantity | Mol. Wt.           | Isotype    |
|--------------------------------------|-----------|----------|--------------------|------------|
| ADAM9 (D64B5) Rabbit mAb             | 4151      | 20 μΙ    | 100-115, 75-80 kDa | Rabbit IgG |
| DLL1 Antibody                        | 2588      | 20 μΙ    | 82 kDa             | Rabbit IgG |
| DLL3 (G93) Antibody                  | 2483      | 20 μΙ    | 65 kDa             | Rabbit IgG |
| DLL4 Antibody                        | 2589      | 20 μΙ    | 75–80 kDa          | Rabbit IgG |
| Jagged1 (28H8) Rabbit mAb            | 2620      | 20 μΙ    | 180 kDa            | Rabbit IgG |
| Jagged2 (C23D2) Rabbit mAb           | 2210      | 20 μΙ    | 150 kDa            | Rabbit IgG |
| Numb (C29G11) Rabbit mAb             | 2756      | 20 μΙ    | 72, 74 kDa         | Rabbit IgG |
| RBPSUH (D10A4) XP® Rabbit mAb        | 5313      | 20 μΙ    | 61 kDa             | Rabbit IgG |
| TACE (D22H4) Rabbit mAb              | 6978      | 20 μΙ    | 135 kDa            | Rabbit IgG |
| Anti-rabbit IgG, HRP-linked Antibody | 7074      | 100 μΙ   |                    | Goat       |

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

**Recommended Antibody Dilutions:** 

Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The Notch Receptor Interaction Antibody Sampler Kit provides an economical means to evaluate Notch signaling. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

**Background:** Notch signaling is activated upon engagement of the Notch receptor with its ligands, the Delta, Serrate, Lag2 (DSL) single-pass type I membrane proteins. DSL proteins contain multiple EGF-like repeats and a DSL domain that is required for binding to Notch (1,2). Five DSL proteins have been identified in mammals: Jagged1, Jagged2, Delta-like (DLL) 1, 3, and 4 (3). Ligand binding to the Notch receptor results in two sequential proteolytic cleavages of the receptor by the ADAM protease and the  $\gamma$ -secretase complex. The intracellular domain of Notch is released and then translocates to the nucleus where it activates transcription. Notch ligands may also be processed in a similar manner, suggesting bi-directional signaling through receptor-ligand interactions

TNF- $\alpha$  converting enzyme (TACE), also known as ADAM17, is a transmembrane metalloprotease that plays a key role in the cleavage of a number cell surface molecules in a process known as "shedding". TACE is abundantly expressed in many adult tissues, but in fetal development, expression is differentially regulated (7). TACE activates Notch in a ligandindependent manner and has been shown to play a role in the development of the *Drosophila* nervous system (8).

Recombining Binding Protein, SUppressor of Hairless (RB-PSUH), also termed RBP-J or CSL, is the DNA-binding component of the transcription complex regulated by canonical Notch signaling. In the absence of Notch activation, RBPSUH suppresses target gene expression through interactions with a co-repressor complex containing histone deacetylase. Upon activation of Notch receptors, the Notch intracellular domain (NICD) translocates to the nucleus and binds to RBPSUH. This displaces the co-repressor complex and replaces it with a transcription activation complex that includes Mastermindlike (MAML) proteins and histone acetylase p300, leading to transcriptional activation of Notch target genes (9-11).

Numb contains an amino-terminal phosphotyrosine-binding (PTB) domain and carboxy-terminal endocytic binding motifs for α-adaptin and EH (Eps15 homology) domain-containing proteins, indicating a role in endocytosis (12,13). There are four mammalian Numb splicing isoforms that are differentially expressed and may have distinct functions (14-16). Numb acts as a negative regulator of Notch signaling by promoting ubiquitination and degradation of Notch (17). The protein is asymmetrically segregated into one daughter cell during cell division, producing two daughter cells with different responses to Notch signaling and different cell fates (18,19).

Specificity/Sensitivity: ADAM9 (D64B5) Rabbit mAb, DLL4 Antibody, Jagged1 (28H8) Rabbit mAb, Jagged2 (C23D2) Rabbit mAb, Numb (C29G11) Rabbit mAb, RBPSUH (D10A4) XP® Rabbit mAb, and TACE (D22H4) Rabbit mAb recognize endogenous levels of total respecitive protein. DLL1 Antibody recognizes only transfected levels of DLL1 protein. It does not recognize transfected levels of rat DLL3 or human DLL4. DLL3 (G93) Antibody recognizes only transfected levels of DLL3 protein. It does not recognize transfected levels of rat DLL1 or human DLL4. Jagged1 (28H8) Rabbit mAb does not cross-react with Jagged2. Jagged2 (C23D2) Rabbit mAb does not cross-react with Jagged1.

Source/Purification: Monoclonal antibodies are produced by immunizing animals either with a recombinant protein specific to the amino terminus of human TACE protein or with a synthetic peptide corresponding to residues surrounding Glu1140 (intracellular region) of human Jagged1 protein, residues surrounding Ala117 of human Jagged2 protein, residues surrounding Ala570 of human Numb protein, or residues near the carboxy terminus of human ADAM9 protein or residues surrounding Gln110 of human RBPSUH protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ala627 of human DLL1 protein, residues surrounding Gly93 of mouse DLL3 protein, residues surrounding Leu617 of human DLL4 protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

U.S. Patent No. 5,675,063

## **Background References:**

- (1) Wilson, A. and Radtke, F. (2006) FEBS Lett 580, 2860-8.
- (2) Hansson, E.M. et al. (2004) Semin Cancer Biol 14, 320-8.
- (3) Chiba, S. (2006) Stem Cells 24, 2437-47.
- (4) Bland, C.E. et al. (2003) *J Biol Chem* 278, 13607-10.
- (5) Six, E. et al. (2003) Proc Natl Acad Sci U S A 100, 7638-43.
- (6) LaVoie, M.J. and Selkoe, D.J. (2003) J Biol Chem 278, 34427-37.
- (7) Black, R.A. et al. (1997) Nature 385, 729-33.
- (8) Delwig, A. and Rand, M.D. (2008) *Cell Mol Life Sci* 65, 2232-43.
- (9) Ehebauer, M. et al. (2006) Sci STKE 2006, cm7.
- (10) Borggrefe, T. and Oswald, F. (2009) *Cell Mol Life Sci* 66, 1631-46.
- (11) Kopan, R. and Ilagan, M.X. (2009) Cell 137, 216-33.
- (12) Berdnik, D. et al. (2002) Dev Cell 3, 221-31.
- (13) Santolini, E. et al. (2000) J Cell Biol 151, 1345-52.
- (14) Dho, S.E. et al. (1999) J Biol Chem 274, 33097-104.
- (15) Verdi, J.M. et al. (1999) Proc Natl Acad Sci U S A 96, 10472-6.
- (16) Verdi, J.M. et al. (1999) *Proc Natl Acad Sci U S A* 96, 10472-6.
- (17) McGill, M.A. and McGlade, C.J. (2003) J Biol Chem 278, 23196-203.
- (18) Verdi, J.M. et al. (1996) Curr Biol 6, 1134-45.
- (19) Reugels, A.M. et al. (2006) Dev Dyn 235, 934-48.

® 2014 Cell Signaling Technology, Inc.