

# SignalSilence® KLHL12 siRNA I



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

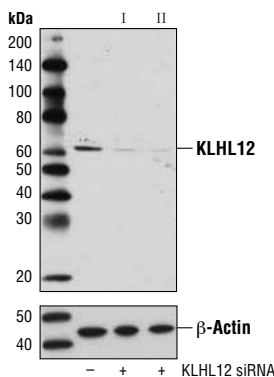
### Species Cross-Reactivity: H, (Mk)

**Description:** SignalSilence® KLHL12 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit KLHL12 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

**Background:** Cullins are proteins that function as molecular scaffolds for modular ubiquitin ligases typified by the SCF (Skp1-CUL1-F-box) complex (1-3). The substrate selectivity of these E3 ligases is dictated by a specificity module that binds cullins. In the SCF complex, this module is composed of Skp1, which binds directly to CUL1, and a member of the F-box family of proteins such as Skp2 (1-4). CUL3 has been shown to be required for embryonic development in mammals and *Caenorhabditis elegans* (5-7) but until recently, its substrate specificity adaptor had yet to be elucidated. It is now recognized that substrate adaptors for CUL3-based ubiquitin ligase complexes contain a conserved BTB/POZ (Pox virus and Zinc finger) domain. This domain, which was initially identified in the *Drosophila* transcriptional repressors broad complex, tramtrack, and bric-a-brac is present in more than 190 human proteins. BTB proteins contain a variety of putative protein-protein interaction domains, including MATH domains, zinc finger repeats, and kelch repeats (8).

There are several lines of evidence suggesting that Kelch-like 12 protein (KLHL12) is a substrate-specific adaptor for the CUL3-based ubiquitin ligase complex. Analysis of the amino acid sequence of KLHL12 reveals an amino-terminal BTB motif, a central linker region, and a carboxy-terminal kelch domain composed of kelch repeats. Furthermore, KLHL12 has been shown to negatively regulate Wnt signaling by binding Disheveled and targeting it for ubiquitin-dependent proteasomal degradation (9). More recently, KLHL12 was shown to drive the assembly of large COPII vesicles by promoting the monoubiquitination of the COPII component Sec31. As a result, CUL3-KLHL12-dependent ubiquitination is essential for collagen export, a step that is required for integrin-dependent mouse embryonic stem cell division (10).

**Specificity/Sensitivity:** KLHL12 siRNA I inhibits human and monkey KLHL12 expression.



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® KLHL12 siRNA I (+), or SignalSilence® KLHL12 siRNA II #9420 (+), using KLHL12 (2G2) Mouse mAb #9406 (upper) or  $\beta$ -Actin (D6A8) Rabbit mAb #8457 (lower). The KLHL12 (2G2) Mouse mAb confirms silencing of KLHL12 expression, while the  $\beta$ -Actin (D6A8) Rabbit mAb is used as a loading control.

**Directions for Use:** CST recommends transfection with 100 nM SignalSilence® KLHL12 siRNA I 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use. Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300  $\mu$ l per well.

**Quality Control:** Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

**Entrez-Gene ID** #59349  
**Swiss-Prot Acc.** #Q53G59

**Storage:** KLHL12 siRNA I is supplied in RNase-free water. Aliquot and store at -20°C.

**Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.**

### Background References:

- (1) Zheng, N. et al. (2002) *Nature* 416, 703-9.
- (2) Skowrya, D. et al. (1997) *Cell* 91, 209-19.
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