Store at -20°C

#4487

RNAi Machinery Antibody Sampler Kit

☑ 1 Kit
(5 x 20 µl)

Cell Signaling TECHNOLOGY®

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Mili (D14F5) XP® Rabbit mAb	5940	20 µl	110 kDa	Rabbit IgG
Drosha (D28B1) Rabbit mAb	3364	20 µl	160 kDa	Rabbit IgG
Argonaute 2 (C34C6) Rabbit mAb	2897	20 µl	97 kDa	Rabbit IgG
Dicer (D38E7) Rabbit mAb	5362	20 µl	220 kDa	Rabbit IgG
Argonaute 1 (D84G10) XP® Rabbit mAb	5053	20 µl	97 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		

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

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 antibody.

Recommended Antibody Dilutions:
Western blotting 1:1000

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

Description: The RNAi Machinery Antibody Sampler Kit provides an economical means to analyze proteins associated with endogenous RNA interference. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: RNA interference (RNAi) serves as a global mechanism of gene regulation in eukaryotes. Through interactions with Dicer, Drosha, Argonaute 2 (Ago2) and Miwi/Mili proteins, microRNA (miRNA) is processed within the nucleus and utilized for gene silencing and down regulation of gene expression. Dicer is a member of the RNase III family that specifically cleaves double-stranded RNA to generate microRNA (miRNA) (1). Long, primary transcripts (pri-miRNAs) are processed to stem-looped pre-miRNAs by the nuclear RNase III Drosha (2) and are then transported to the cytoplasm for further processing by Dicer to produce mature, 22-nucleotide miRNAs (3). The mature miRNA then becomes a part of the RNA-Induced Silencing Complex (RISC) and can bind to the 3' UTR of the target mRNA (3). Interference of Drosha pri-miRNA processing results in the increase of pri-miRNAs and the decrease of pre-miRNAs (2). Drosha forms part of a multiprotein complex called the Microprocessor along with other components, such as DGCR8 (4). Both Drosha and DGCR8 are necessary for miRNA biogenesis (5). Argonaute protein family members participate in various steps of miRNA-mediated gene silencing such as repression of translation and mRNA turnover (6). The *Drosophila* piwi gene was identified as being required for the self-renewal of germ-line stem cells, and its homologues are well conserved among various species including *Arabidopsis*, *C. elegans* and human (7). Miwi and Mili proteins are both mouse homologs of Piwi and contain a carboxy-terminal Piwi domain that binds to Piwi-interacting RNAs (piRNAs) in male germ cells and are essential for spermatogenesis in mouse (8-11).

Specificity/Sensitivity: Each antibody in the RNAi Machinery Antibody Sampler Kit recognizes endogenous levels of their target protein and does not cross-react with other proteins.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to the residues surrounding Ala1123 of human Dicer or residues surrounding Leu118 of mouse Mili protein. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with synthetic peptides corresponding to the sequence of mouse Argonaute 2 or around Gly953 of human Drosha proteins.

Background References:

(1) Hutvágner, G. and Zamore, P.D. (2002) *Science* 297, 2056-60.

(2) Lee, Y. et al. (2003) *Nature* 425, 415-9.

(3) Diederichs, S. and Haber, D.A. (2007) *Cell* 131, 1097-108.

(4) Denli, A.M. et al. (2004) *Nature* 432, 231-5.

(5) Gregory, R.I. et al. (2004) *Nature* 432, 235-40.

(6) Peters, L. and Meister, G. (2007) *Mol Cell* 26, 611-23.

(7) Cox, D.N. et al. (1998) *Genes Dev* 12, 3715-27.

(8) Kuramochi-Miyagawa, S. et al. (2001) *Mech Dev* 108, 121-33.

(9) Aravin, A. et al. (2006) *Nature* 442, 203-7.

(10) Grivna, S.T. et al. (2006) *Proc Natl Acad Sci U S A* 103, 13415-20.

(11) Grivna, S.T. et al. (2006) *Genes Dev* 20, 1709-14.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#4487

Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween®20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween®20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween®20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween®20 (100%).
10. **Phototope®-HRP Western Blot Detection System** #7071**:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.