Anti-PIWIL2 Antibody [PSH27-16]

HA724677

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Mouse, Rat
Applications:	WB, IHC-Fr, IHC-P, IP, mIHC
Molecular Wt:	Predicted band size: 109 kDa
Clone number:	PSH27-16

Description: Endoribonuclease that plays a central role during spermatogenesis by repressing transposable elements and preventing their mobilization, which is essential for the germline integrity (By similarity). Plays an essential role in meiotic differentiation of spermatocytes, germ cell differentiation and in self-renewal of spermatogonial stem cells (By similarity). Acts via the piRNA metabolic process, which mediates the repression of transposable elements during meiosis by forming complexes composed of piRNAs and Piwi proteins and govern the methylation and subsequent repression of transposons (By similarity). During piRNA biosynthesis, plays a key role in the piRNA amplification loop, also named ping-pong amplification cycle, by acting as a 'slicer-competent' piRNA endoribonuclease that cleaves primary piRNAs, which are then loaded onto 'slicer-incompetent' PIWIL4 (By similarity). PIWIL2 slicing produces a pre-miRNA intermediate, which is then processed in mature piRNAs, and as well as a 16 nucleotide by-product that is degraded (By similarity). Required for PIWIL4/MIWI2 nuclear localization and association with secondary piRNAs antisense (By similarity). Besides their function in transposable elements repression, piRNAs are probably involved in other processes during meiosis such as translation regulation (By similarity). Indirectly modulates expression of genes such as PDGFRB, SLC2A1, ITGA6, GJA7, THY1, CD9 and STRA8 (By similarity). When overexpressed, acts as an oncogene by inhibition of apoptosis and promotion of proliferation in tumors. Represses circadian rhythms by promoting the stability and activity of core clock components BMAL1 and CLOCK by inhibiting GSK3B-mediated phosphorylation and ubiquitination-dependent degradation of these proteins.

Immunogen:	Recombinant protein within mouse PIWIL2 aa 1-250.
Positive control:	Rat testis tissue lysate, Mouse testis tissue lysate.
Subcellular location:	Cytoplasm.
Database links:	SwissProt: Q8CDG1 Mouse Entrez Gene: 306011 Rat
Recommended Dilutions:	
WB	1:2,000
IHC-Fr	1:250
IHC-P	1:500
IP	1-2μg/sample
mIHC	1:1,000
Storage Buffer:	1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

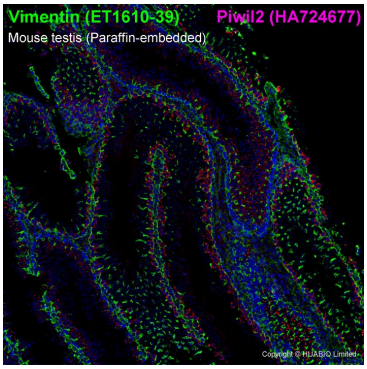


Fig1: mIHC analysis of mouse testis tissue (paraffin-embedded) with PIWIL2 (HA724677, 1/1,000) and Vimentin (ET1610-39, 1/10,000).

The immunostaining was performed with the IRISKitCmTSA Kit (900802). DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

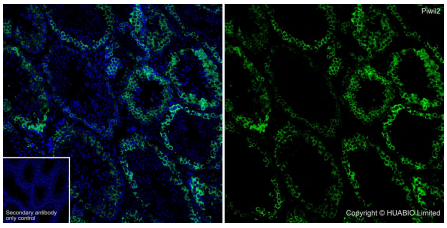


Fig2: Application: Immunofluorescence (IHC-Fr)

Species: Mouse
Tissue: Testis
Sample: Frozen section

Antigen retrieval: Not required

Wash buffer: 1× TBST
Blocking: 10% normal goat serum + 1% Triton X-100 + 0.3 M Glycine in TBST, 30 minutes at room temperature.

Primary antibody: HA724677, 1/250, overnight at 4℃.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 1.5 hours at room temperature.

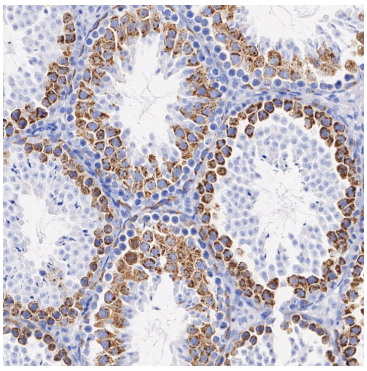


Fig3: Application: Immunohistochemistry (IHC-P)

Species: Mouse
Tissue: Testis
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA724677, 1/500, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

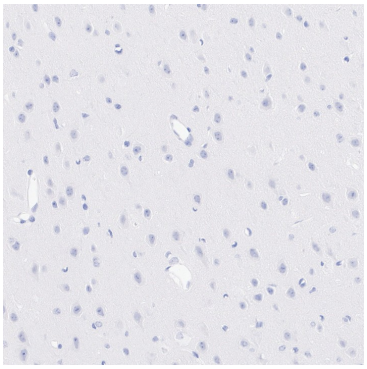


Fig4: Application: Immunohistochemistry (IHC-P)

Species: Mouse
Tissue: Brain
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA724677, 1/500, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

Negative expression of PIWIL2 protein in brain is consistent with the predicted expression pattern.

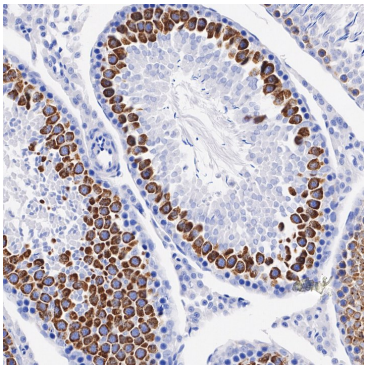


Fig5: Application: Immunohistochemistry (IHC-P)

Species: Rat
Tissue: Testis
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA724677, 1/500, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

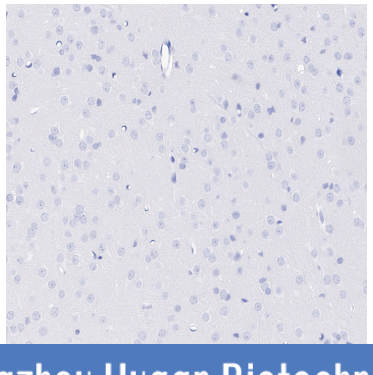


Fig6: Application: Immunohistochemistry (IHC-P)

Species: Rat
Tissue: Brain
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.

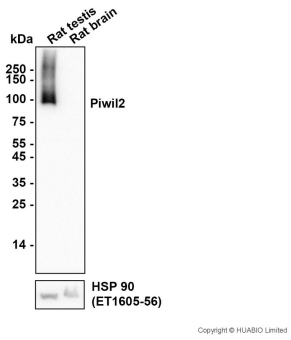


Fig7: Western blot analysis of PIWIL2 on different lysates with Rabbit anti-PIWIL2 antibody (HA724677) at 1/2,000 dilution.

Lane 1: Rat testis tissue lysate
Lane 2: Rat brain tissue lysate

Negative control: Brain (PMID: 16377660).

Lysates/proteins at 30 µg/Lane.
Exposure time: 20 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: HA724677, 1/2,000 in primary antibody dilution buffer (K1803), overnight at 4 °C
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

Predicted band size: 109 kDa
Observed band size: 109 kDa

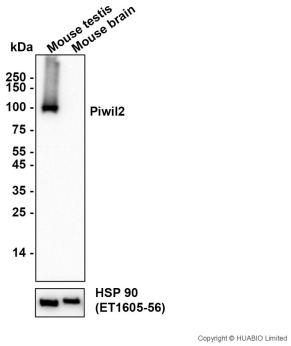


Fig8: Western blot analysis of PIWIL2 on different lysates with Rabbit anti-PIWIL2 antibody (HA724677) at 1/2,000 dilution.

Lane 1: Mouse testis tissue lysate
Lane 2: Mouse brain tissue lysate

Negative control: Brain (PMID: 16377660).

Lysates/proteins at 30 µg/Lane.
Exposure time: 20 seconds; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: HA724677, 1/2,000 in primary antibody dilution buffer (K1803), overnight at 4 °C
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

Predicted band size: 109 kDa
Observed band size: 109 kDa

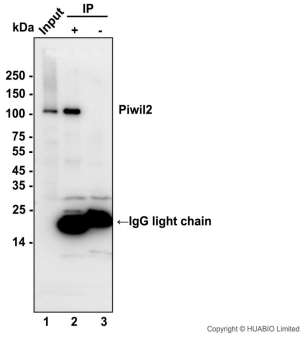


Fig9: Immunoprecipitation (IP)

PIWIL2 was immunoprecipitated in 0.2 mg mouse testis tissue lysate with HA724677 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA724677 at 1/5,000 dilution. HRP Conjugated Rabbit IgG kappa light chain Mouse Monoclonal Antibody (M1208-2) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Mouse testis tissue lysate (input)
Lane 2: HA724677 IP in mouse testis tissue lysate
Lane 3: Rabbit IgG instead of HA724677 in mouse testis tissue lysate

Exposure time: 1 minute 50 seconds
Blocking: 5% NFDM/TBST, 1 hour at room temperature

Primary dilution: HA724677, 1/2,000 in primary antibody dilution buffer (K1803), 2 hours at room temperature

Predicted band size: 109 kDa
Observed band size: 109 kDa

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Li Y et al. PIWIL2/PDK1 Axis Promotes the Progression of Cervical Epithelial Lesions via Metabolic Reprogramming to Maintain Tumor-Initiating Cell Stemness. Adv Sci (Weinh). 2024 Dec
2. Hammad G et al. HEPPAR1 and PIWIL2 as Panel Markers for Hepatocellular Carcinoma. Asian Pac J Cancer Prev. 2024 Jun