

Anti-SLA (haploid sperm cell-specific antigen) antibody, clone TRA54

73-001 100 ug

Shipment and storage temperature: Ship at 4°C and store at -20°C

Reactivity: Mouse and rat. Not tested for other species.

Immunogen: Cell lysates of adult mouse testes

Applications:

1. Western blotting (~1 ug/ml)

- 2. Immunohistochemical staining
- 3. Immuno-electron microscopy (Ref. 3). 4. Immunoprecipitation

Product: The antibody was purified from the serum-free culture medium of the hybridoma clone TRA54 by proprietary chromatography processes under mild conditions.

Form: Purified rat IgM (1 mg/ml) in PBS(-), 50% glycerol

Background: Many transcripts for proteins specifically expressed in haploid germ cells are synthesized in the spermatogenesis. A monoclonal antibody TRA54 recognizes specific organelles in germ cell cytoplasm from spermatocytes to spermatids. Western blots using TRA54 revealed broad bands with 85 kD, 190 kD and >200 kDa in testis probably due to alternative splicing and/or post-translational modification (Fig. 1). The molecular size diversity is due to the difference in glycosilation.

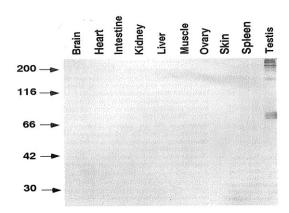
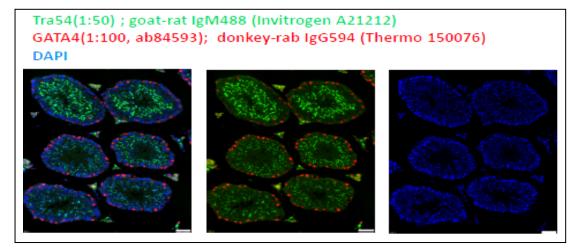


Fig.1. Western blot analysis of various mouse tissues with TRA54 antibody.

For each tissue homogenate, 100 ug protein was applied to each lane. Antibody was used at 1 ug/ml.



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Fig. 2. Immunohistochemical staining of mouse testicular testia with TRA54 antibody.

Sections of 8-month mouse testis were fixed with 4% paraformaldehyde and embedded in paraffin. After antigen retrieval by exposing to steam for 15 min in 10 mM citrate buffer (pH 6), they were reacted with the anti-TRA54 antibody (Green) at 1/50 dilution and anti-GATA4 antibody (Red). As the 2nd antibody, Donky anti-rat Alexa Fluor488 and Donkey anti-rabbit Alexa Fluor594 were used. DNA was stained with DAPI (Blue).

Anti-GATA antibody stains nuclei of Sertoli cells in seminiferous tubule while anti-TRA54 antibody stains reproductive cells.

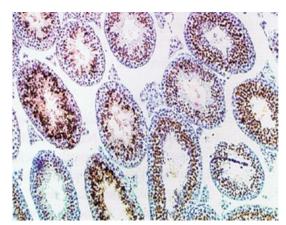


Fig. 3. Immunohistochemical staining of mouse testicular cross-section with TRA54 antibody.

Testis from C57BL/6 adult mouse was fixed in Bouin's solution, followed by paraffin embedding and sectioning. After deparaffinized, sections were treated with normal goat serum, reacted with TRA54 antibody and incubated with biotinylated sheep anti-rat Ig antibody and biotin peroxydase/avidin, followed by incubation with hydrogen peroxide and diaminobenzidine.

Related product: 73-003 Anti-Gena (testicular germ cell specific antigen) antibody, clone TRA98

References: Antibody TRA54 has been used in the following publications (12). Matsubara Y. et al. TALEN-Mediated Gene Disruption on Y Chromosome Reveals Critical Role of EIF2S3Y in Mouse Spermatogenesis. Stem Cells Dev. 2015 May 15;24(10):1164-70. PMID:25579647.IHC-P (mouse)

Okuda H. et al. A novel transcriptional factor Nkapl is a germ cell-specific suppressor of Notchsignaling and is indispensable for spermatogenesis. <u>PLoS One.</u> 2015 Apr 14;10(4):e0124293. PMID:25875095. **WB (mouse)**

Park ASK et al. Targeted disruption of Tbc1d20 with zinc-finger nucleases causes cataracts and testicular abnormalities in mice. <u>BMC Genet.</u> 2014 Dec 5;15:135. PMID: 25476608. **IHC-P (mouse)**

Arroteia KF et al. Albumin is synthesized in epididymis and aggregates in a high molecular mass glycoprotein complex involved in sperm-egg fertilization. PLoS One. 2014 Aug 1;9(8):e103566. PMID: 25084016. WB, Dot blot, IP, IF, Immuno Electron Microscopy (mouse)

Liegel RP et al. Alkylglycerone phosphate synthase (AGPS) deficient mice: models for rhizomelic chondrodysplasia punctate type 3 (RCDP3) malformation syndrome.

<u>Mol Genet Metab Rep.</u> 2014;1:299-311. PMID: 25197626.**IHC-P (mouse)**

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- Tokunaga K et al. The 193-Base Pair *Gsg2* (Haspin) Promoter Region Regulates Germ Cell-Specific Expression Bidirectionally and Synchronously. <u>Biol Reprod.</u> 2007 Mar;76(3):407-14. PMID:17123944. **WB (mouse)**
- Tokuhiro K et al. The 193-base pair Gsg2 (haspin) promoter region regulates germ cell-specific expression bidirectionally and synchronously. <u>Biol Reprod.</u> 2007 Mar;76(3):407-14. PMID: 17123944. **WB (mouse)**
- Arrotéia KF et al Identification and characterization of an antigen recognized by monoclonal antibody TRA 54 in mouse epididymis and vas deferens J Androl 25: 914-921 (2004) PMID: <u>15477363</u> **WB, IHC (mouse)**
- Matsuoka Y et al. Cloning and characterization of a mouse spergen-1 localized in sperm mitochondria. Int J Androl. 2004 Jun;27(3):152-60. PMID: 15139970 **WB (mouse)**
- Ventelä S et al "Intercellular organelle traffic through cytoplasmic bridges in early spermatids of therat: mechanisms of haploid gene product sharing" *Mol Biol Cell* 14: 2768-2780 (2003) PMID: 12857863 IHC, Immno-Electron Microscopy.(mouse)
- Deng W, Lin H. miwi, a murine homolog of piwi, encodes a cytoplasmic protein essential for spermatogenesis. <u>Dev Cell.</u> 2002 Jun;2(6):819-30. PMID: 12062093.**IHC-F (mouse)**
- Pereira LA et al Characterization and expression of a stage specific antigen by monoclonal antibody TRA 54 in testicular germ cell Int J Androl 21: 34-40 (1998) PMID: 9639150 WB, IHC (mouse)