

# Anti-TIM 3 Antibody [PSH16-50] - BSA and Azide free

## HA751599



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IHC-P, IP
<b>Molecular Wt:</b>	Predicted band size: 33 kDa
<b>Clone number:</b>	PSH16-50

**Description:** Hepatitis A virus cellular receptor 2 (HAVCR2), also known as T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), is a protein that in humans is encoded by the HAVCR2 (TIM-3) gene. HAVCR2 was first described in 2002 as a cell surface molecule expressed on IFN $\gamma$  producing CD4<sup>+</sup> Th1 and CD8<sup>+</sup> Tc1 cells. Later, the expression was detected in Th17 cells, regulatory T-cells, and innate immune cells (dendritic cells, NK cells, monocytes, macrophages). HAVCR2 receptor is a regulator of the immune response.

**Positive control:** RPMI 8226 cell lysate, RAW264.7 cell lysates, human lung cancer tissue, mouse spleen tissue, rat spleen tissue.

**Subcellular location:** Membrane, Cell junction, Cell membrane.

**Database links:** SwissProt: Q8TDQ0 Human | Q8VIM0 Mouse | P0C0K5 Rat

**Recommended Dilutions:**

<b>WB</b>	1:2,000
<b>IHC-P</b>	1:200-1:1,000
<b>IP</b>	1-2 $\mu$ g/sample

**Storage Buffer:** 1\*PBS (pH7.4).

**Storage Instruction:** Store at +4 $^{\circ}$ C after thawing. Aliquot store at -20 $^{\circ}$ C. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

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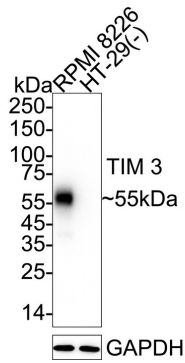
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## Images



**Fig1:** Western blot analysis of TIM 3 on different lysates with Rabbit anti-TIM 3 antibody (HA751599) at 1/2,000 dilution.

Lane 1: RPMI 8226 cell lysate (20 µg/Lane)

Lane 2: HT-29 cell lysate (negative) (20 µg/Lane)

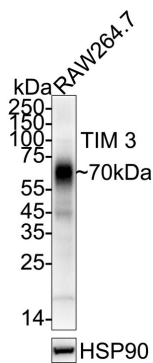
Predicted band size: 33 kDa

Observed band size: 55 kDa

Exposure time: 5 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDm/TBST for 1 hour at room temperature. The primary antibody (HA751599) at 1/2,000 dilution was used in primary antibody dilution (K1803) at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



**Fig2:** Western blot analysis of TIM 3 on RAW264.7 cell lysates with Rabbit anti-TIM 3 antibody (HA751599) at 1/2,000 dilution.

Lysates/proteins at 20 µg/Lane.

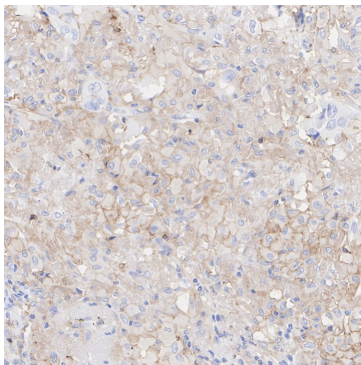
Predicted band size: 33 kDa

Observed band size: 70 kDa

Exposure time: 1 minute; ECL: K1802;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDm/TBST for 1 hour at room temperature. The primary antibody (HA751599) at 1/2,000 dilution was used in 5% NFDm/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.



**Fig3:** Immunohistochemical analysis of paraffin-embedded human lung cancer tissue with Rabbit anti-TIM 3 antibody (HA751599) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA751599) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

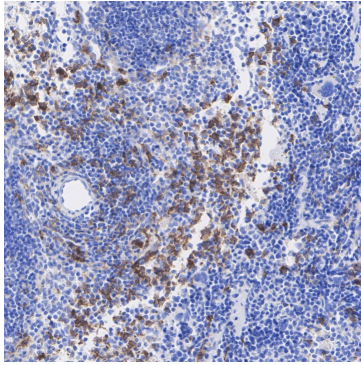
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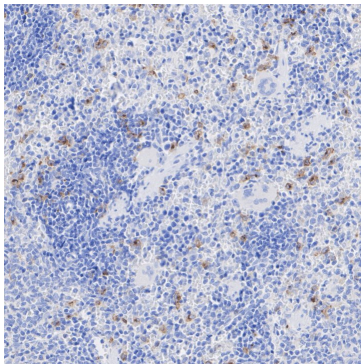
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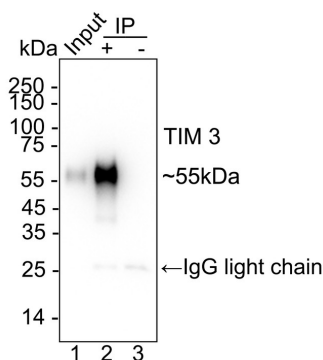
**Fig4:** Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-TIM 3 antibody (HA751599) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA751599) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig5:** Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-TIM 3 antibody (HA751599) at 1/200 dilution.

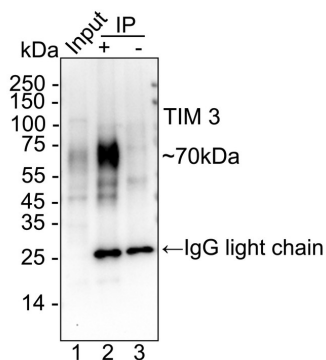
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (HA751599) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig6:** TIM 3 was immunoprecipitated from 0.2 mg RPMI 8226 cell lysate with HA751599 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751599 at 1/2,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: RPMI 8226 cell lysate (input)  
 Lane 2: HA751599 IP in RPMI 8226 cell lysate  
 Lane 3: Rabbit IgG instead of HA751599 in RPMI 8226 cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)  
 Exposure time: 3 seconds; ECL: K1801



**Fig7:** TIM 3 was immunoprecipitated from 0.2 mg RAW264.7 cell lysate with HA751599 at 2  $\mu$ g/10  $\mu$ l beads. Western blot was performed from the immunoprecipitate using HA751599 at 1/2,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: RAW264.7 cell lysate (input)

Lane 2: HA751599 IP in RAW264.7 cell lysate

Lane 3: Rabbit IgG instead of HA751599 in RAW264.7 cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)

Exposure time: 46 seconds; ECL: K1802

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

### Background References

1. Kandel S et al. The TIM3/GaI9 signaling pathway: An emerging target for cancer immunotherapy. *Cancer Lett.* 2021 Jul
2. Palacios LM et al. TIM3 Expression in Anaplastic-Thyroid-Cancer-Infiltrating Macrophages: An Emerging Immunotherapeutic Target. *Biology (Basel).* 2022 Nov

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