# Anti-CD168 Antibody [PSH02-16] - BSA and Azide free HA750786



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human

Applications: WB, IF-Cell, IHC-P, FC

Molecular Wt: Predicted band size: 84 kDa

Clone number: PSH02-16

**Description:** The protein encoded by this gene is involved in cell motility. It is expressed in breast tissue

and together with other proteins, it forms a complex with BRCA1 and BRCA2, thus is potentially associated with higher risk of breast cancer. Alternatively spliced transcript variants encoding different isoforms have been noted for this gene. Receptor for hyaluronic acid (HA). Involved in cell motility. When hyaluronan binds to HMMR, the phosphorylation of a number of proteins, including PTK2/FAK1 occurs. May also be involved in cellular transformation and metastasis formation, and in regulating extracellular-regulated kinase

(ERK) activity. May act as a regulator of adipogenisis.

Immunogen: Recombinant protein within human CD168 aa 1-350 / 724 (O75330).

Positive control: K-562 cell lysate, SW480 cell lysate, HepG2 cell lysate, Raji cell lysate, MCF7 cell lysate,

SK-Br-3 cell lysate, HeLa cell lysate, Jurkat cell lysate, HeLa, human colon carcinoma

tissue, human testis tissue, human tonsil tissue.

**Subcellular location:** Cell surface, Cytoplasm, cytoskeleton, spindle.

Database links: SwissProt: 075330 Human

**Recommended Dilutions:** 

WB 1:1,000 IF-Cell 1:100 IHC-P 1:1,000 FC 1:1,000

Storage Buffer: 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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#### **Images**

kDa+ 250-150-150-100-37-25-20-15-10-GAPDH Fig1: Western blot analysis of CD168 on different lysates with Rabbit anti-CD168 antibody (HA750786) at 1/1,000 dilution.

Lane 1: K-562 cell lysate Lane 2: SW480 cell lysate Lane 3: HepG2 cell lysate Lane 4: Raji cell lysate Lane 5: MCF7 cell lysate Lane 6: SK-Br-3 cell lysate Lane 7: HeLa cell lysate Lane 8: Jurkat cell lysate

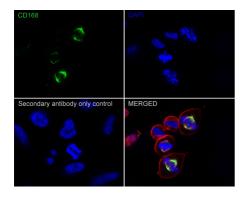
Lysates/proteins at 20 µg/Lane.

Predicted band size: 84 kDa Observed band size: 84 kDa

Exposure time: 1 minute 46 seconds;

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 (HA750786) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

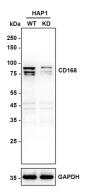


**Fig2:** Immunocytochemistry analysis of HeLa cells labeling CD168 with Rabbit anti-CD168 antibody (HA750786) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-CD168 antibody (HA750786) at 1/100 dilution in 1% BSA in PBST overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at  $+4^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor  $\pm$  594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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**Fig3:** Western blot analysis of CD168 on different lysates with Rabbit anti-CD168 antibody (HA750786) at 1/2,000 dilution.

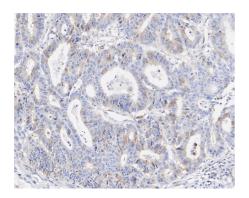
Lane 1: HAP1-parental cell lysate Lane 2: HAP1-CD168 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 84 kDa Observed band size: 84 kDa

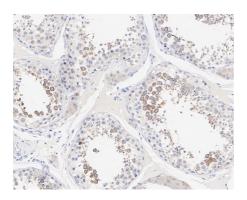
Exposure time: 15 seconds; ECL: K1801;

4-20% SDS-PAGE gel.



**Fig4:** Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-CD168 antibody (HA750786) 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 $_2$ O and PBS, and then probed with the primary antibody (HA750786) 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.

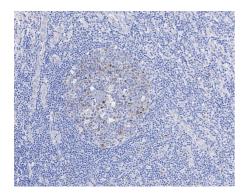


**Fig5:** Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-CD168 antibody (HA750786) at 1/1,000 dilution.

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 (HA750786) 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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**Fig6:** Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-CD168 antibody (HA750786) 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 (HA750786) 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.

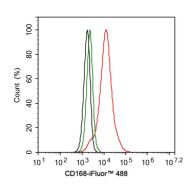


Fig7: Flow cytometric analysis of HeLa cells labeling CD168.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750786, 1µg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4  $^{\circ}$ C for an hour, the cells were stained with a iFluor 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4  $^{\circ}$ C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

#### **Background References**

- Zhu SW et al. Overexpression of CD168 is related to poor prognosis in oral squamous cell carcinoma. Oral Dis. 2022
   Mar
- 2. Zhao HC et al. CD168+ macrophages promote hepatocellular carcinoma tumor stemness and progression through TOP2A/β-catenin/YAP1 axis. iScience. 2023 May