

# Anti-PUMA Antibody [SR42-09]

ET1602-24



<b>Product Type:</b>	Recombinant Rabbit monoclonal IgG, primary antibodies
<b>Species reactivity:</b>	Human, Mouse, Rat
<b>Applications:</b>	WB, IF-Cell, IF-Tissue, IHC-P, FC
<b>Molecular Wt:</b>	Predicted band size: 21 kDa
<b>Clone number:</b>	SR42-09

**Description:** The p53 upregulated modulator of apoptosis (PUMA) also known as Bcl-2-binding component 3 (BBC3), is a pro-apoptotic protein, member of the Bcl-2 protein family. In humans, the Bcl-2-binding component 3 protein is encoded by the BBC3 gene. The expression of PUMA is regulated by the tumor suppressor p53. PUMA is involved in p53-dependent and -independent apoptosis induced by a variety of signals, and is regulated by transcription factors, not by post-translational modifications. After activation, PUMA interacts with antiapoptotic Bcl-2 family members, thus freeing Bax and/or Bak which are then able to signal apoptosis to the mitochondria. Following mitochondrial dysfunction, the caspase cascade is activated ultimately leading to cell death.

**Immunogen:** Synthetic peptide within Human PUMA aa 153-193 / 193.

**Positive control:** HeLa cell lysate, HepG2 cell lysate, HDLM-2 cell lysate, NIH/3T3 cell lysate, Rat stomach tissue lysate, Rat liver tissue lysate, Hela, SKOV-3, human stomach cancer tissue, human small intestine tissue, mouse small intestine tissue, rat small intestine tissue, rat stomach tissue.

**Subcellular location:** Mitochondrion.

**Database links:** SwissProt: Q9BXH1 Human | Q99ML1 Mouse | Q80ZG6 Rat

**Recommended Dilutions:**

<b>WB</b>	1:500-1:2,000
<b>IF-Cell</b>	1:50
<b>IF-Tissue</b>	1:200-1:500
<b>IHC-P</b>	1:2,000-1:30,000
<b>FC</b>	1:50

**Storage Buffer:** 1\*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Shipped at 4°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

**Purity:** Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

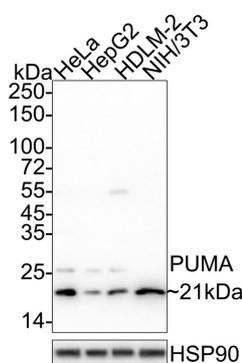
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## Images

**Fig1:** Western blot analysis of PUMA on different lysates with Rabbit anti-PUMA antibody (ET1602-24) at 1/2,000 dilution.

Lane 1: HeLa cell lysate  
Lane 2: HepG2 cell lysate  
Lane 3: HDLM-2 cell lysate  
Lane 4: NIH/3T3 cell lysate



Lysates/proteins at 20 µg/Lane.

Predicted band size: 21 kDa  
Observed band size: 21 kDa

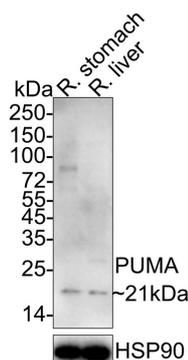
Exposure time: 24 seconds; ECL: K1801;

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 (ET1602-24) 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.

**Fig2:** Western blot analysis of PUMA on different lysates with Rabbit anti-PUMA antibody (ET1602-24) at 1/1,000 dilution.

Lane 1: Rat stomach tissue lysate  
Lane 2: Rat liver tissue lysate



Lysates/proteins at 40 µg/Lane.

Predicted band size: 21 kDa  
Observed band size: 21 kDa

Exposure time: 3 minutes; 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 (ET1602-24) at 1/1,000 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.

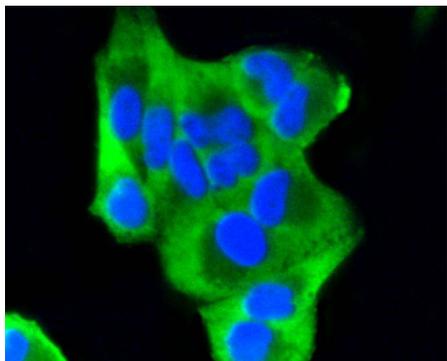
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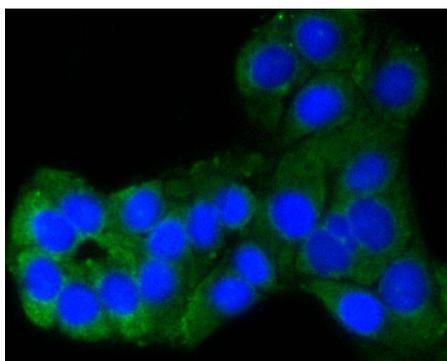
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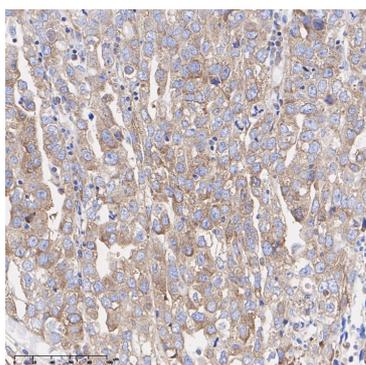
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**Fig3:** ICC staining of PUMA in HeLa cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1602-24, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

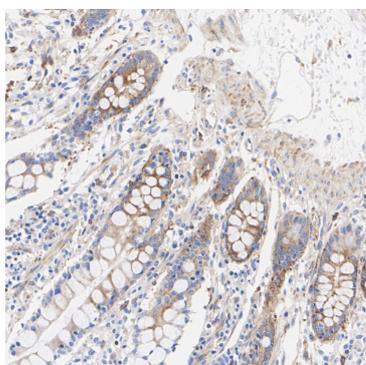


**Fig4:** ICC staining of PUMA in SKOV-3 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1602-24, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



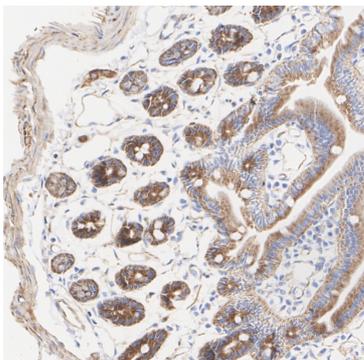
**Fig5:** Immunohistochemical analysis of paraffin-embedded human stomach cancer tissue with Rabbit anti-PUMA antibody (ET1602-24) at 1/5,000 dilution.

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 (ET1602-24) at 1/5,000 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.



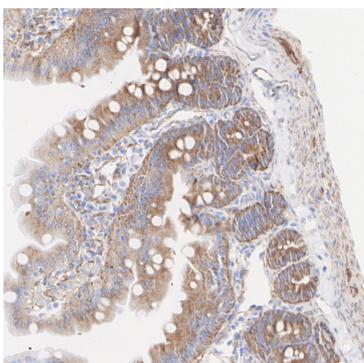
**Fig6:** Immunohistochemical analysis of paraffin-embedded human small intestine tissue with Rabbit anti-PUMA antibody (ET1602-24) at 1/10,000 dilution.

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 (ET1602-24) at 1/10,000 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.



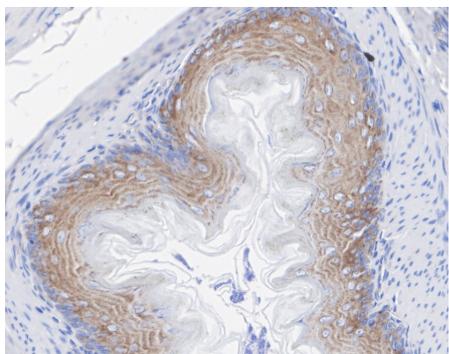
**Fig7:** Immunohistochemical analysis of paraffin-embedded mouse small intestine tissue with Rabbit anti-PUMA antibody (ET1602-24) at 1/30,000 dilution.

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 (ET1602-24) at 1/30,000 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.



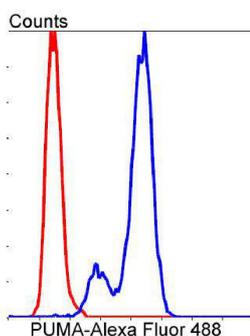
**Fig8:** Immunohistochemical analysis of paraffin-embedded rat small intestine tissue with Rabbit anti-PUMA antibody (ET1602-24) at 1/30,000 dilution.

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 (ET1602-24) at 1/30,000 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.



**Fig9:** Immunohistochemical analysis of paraffin-embedded rat stomach tissue with Rabbit anti-PUMA antibody (ET1602-24) at 1/2,000 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 (ET1602-24) at 1/2,000 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.



**Fig10:** Flow cytometric analysis of PUMA was done on HeLa cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1602-24, 1/50) (blue). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes. Unlabelled sample was used as a control (cells without incubation with primary antibody; red).

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

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### Background References

1. Pace J et al. PuMA: A papillomavirus genome annotation tool. *Virus Evol.* 2020 Aug
2. Kennedy li DE et al. Contribution of Puma to Inflammatory Resolution During Early Pneumococcal Pneumonia. *Front Cell Infect Microbiol.* 2022 May

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