# Anti-Phospho-POLR2A (S5) Antibody [JM51-21] ET1703-87

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| **Product Type:** | Recombinant Rabbit monoclonal IgG, primary antibodies |
| **Species reactivity:** | Human, Mouse, Rat |
| **Applications:** | WB, IP, IF-Cell, IF-Tissue, IHC-P, FC |
| **Molecular Wt:** | Predicted band size: 217 kDa |
| **Clone number:** | JM51-21 |

**Description:** DNA-directed RNA polymerase II subunit RPB1, also known as RPB1, is an enzyme that is encoded by the POLR2A gene in humans. This gene encodes the largest subunit of RNA polymerase II, the polymerase responsible for synthesizing messenger RNA in eukaryotes. The product of this gene contains a carboxy terminal domain composed of heptapeptide repeats that are essential for polymerase activity. These repeats contain serine and threonine residues that are phosphorylated in actively transcribing RNA polymerase. In addition, this subunit, in combination with several other polymerase subunits, forms the DNA-binding domain of the polymerase, a groove in which the DNA template is transcribed into RNA.

**Immunogen:** Synthetic phospho-peptide corresponding to residues surrounding Ser5 of Human POLR2A aa 1590-1627 / 1970.

**Positive control:** HeLa cell lysate, A431 cell lysate, MCF7 cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, HeLa, human kidney tissue, mouse kidney tissue, rat kidney tissue.

**Subcellular location:** Nucleus, Cytoplasm, Chromosome.

**Database links:** SwissProt: P24928 Human | P08775 Mouse
Entrez Gene: 363633 Rat

**Recommended Dilutions:**

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| **WB** | 1:2,000 |
| **IF-Cell** | 1:500 |
| **IHC-P** | 1:200-1:1000 |
| **FC** | 1:1000 |

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

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

**Purity:** Protein A affinity purified.

## Images

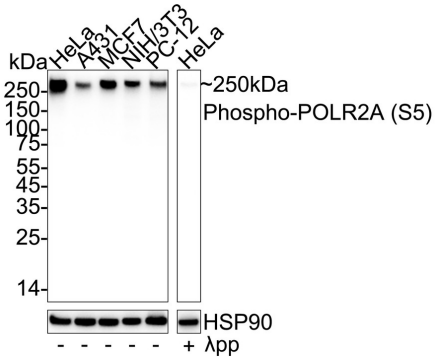


**Fig1:** Western blot analysis of Phospho-POLR2A (S5) on different lysates with Rabbit anti-Phospho-POLR2A (S5) antibody (ET1703-87) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: A431 cell lysate
Lane 3: MCF7 cell lysate
Lane 4: NIH/3T3 cell lysate
Lane 5: PC-12 cell lysate
Lane 6: HeLa cell lysate, the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 217 kDa
Observed band size: 250 kDa

Exposure time: 17 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 (ET1703-87) at 1/50,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

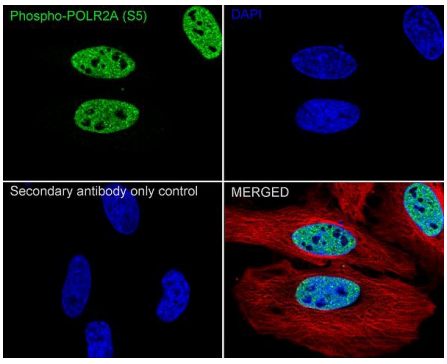


**Fig2:** Immunocytochemistry analysis of HeLa cells labeling Phospho-POLR2A (S5) with Rabbit anti-Phospho-POLR2A (S5) antibody (ET1703-87) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Phospho-POLR2A (S5) antibody (ET1703-87) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. 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 (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

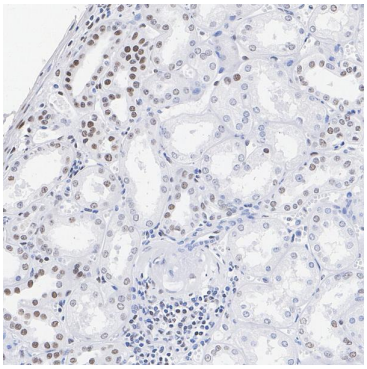

**Fig3:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Phospho-POLR2A (S5) antibody (ET1703-87) at 1/200 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (ET1703-87) 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.

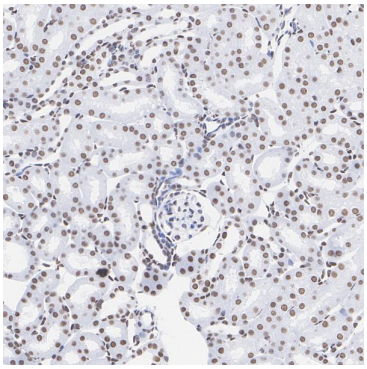

**Fig4:** Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Phospho-POLR2A (S5) antibody (ET1703-87) at 1/1,000 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (ET1703-87) 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.

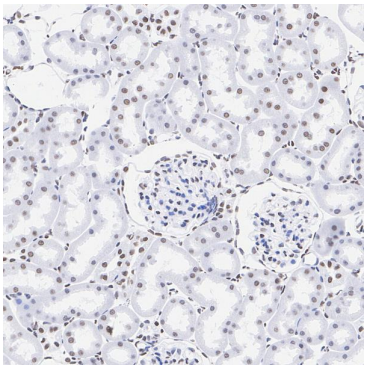

**Fig5:** Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Phospho-POLR2A (S5) antibody (ET1703-87) at 1/1,000 dilution.

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 $ddH_2O$ and PBS, and then probed with the primary antibody (ET1703-87) 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.

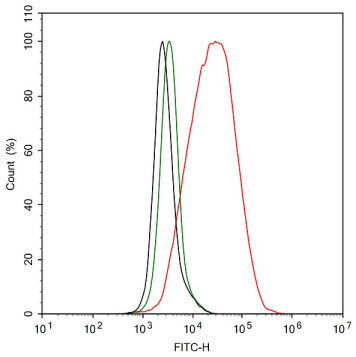


**Fig6:** Flow cytometric analysis of Phospho-POLR2A (S5) was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1703-87, 1/1000) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.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

1. Zhang D et al. USP10 Inhibits Ferroptosis via Deubiquinating POLR2A in Head and Neck Squamous Cell Carcinoma. Adv Sci (Weinh). 2025 Sep
2. Liu C et al. POLR2A blocks osteoclastic bone resorption and protects against osteoporosis by interacting with CREB1. J Cell Physiol. 2021 Jul