Anti-GFP Antibody [SY0243]

ET1607-31

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Species independent
Applications:	WB, IF-Cell, IF-Tissue, IP, IHC-P
Molecular Wt:	Predicted band size: 27 kDa
Clone number:	SY0243

Description: The green fluorescent protein (GFP) was originally identified as a protein involved in the bioluminescence of the jellyfish Aequorea victoria. GFP cDNA produces a fluorescent product when expressed in prokaryotic cells, without the need for exogenous substrates or cofactors, making GFP a useful tool for monitoring gene expression and protein localization in vivo. Several GFP mutants have been developed, including EGFP, which fluoresce more intensely than the wildtype GFP and have shifted excitation maxima, making them useful for FACS and fluorescence microscopy as well as double-labeling applications. GFP is widely used in expression vectors as a fusion protein tag, allowing expression and monitoring of heterologous proteins fused to GFP.

Immunogen: Synthetic peptide within Aequorea victoria GFP aa 1-50 / 238.

Positive control: GFP recombinant protein.

Database links: SwissProt: P42212 AequoreaVictoria

Recommended Dilutions:

WB	1:20,000-1:50,000
IF-Cell	1:500-1:1,000
IF-Tissue	1:500-1:2,000
IP	2-5 μg/ml.
IHC-P	1:5,000

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

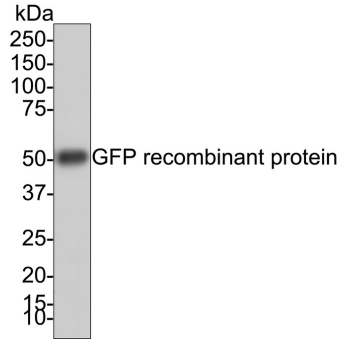

Fig1: Western blot analysis of GFP on GFP recombinant protein with Rabbit anti-GFP antibody (ET1607-31) at 1/20,000 dilution.

Lysates/proteins at 50 ng/Lane.

Exposure time: 30 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 (ET1607-31) at 1/20,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

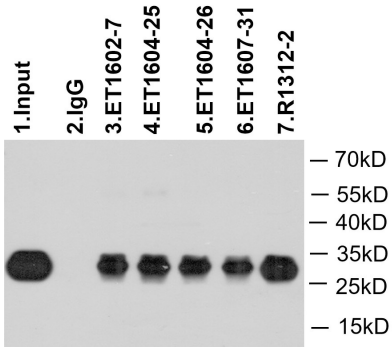

Fig2: GFP tag was immunoprecipitated in 5μg GFP Tag fusion protein lysate with ET1607-31 at 2 μg/20 μl agarose. Western blot was performed from the immunoprecipitate using M1004-8 at 1/10,000 dilution. Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1:20,000 dilution was used for 60 mins at room temperature.

Lane 1: GFP Tag fusion protein lysate (input).
Lane 2: Rabbit IgG instead of ET1607-31 in GFP Tag fusion protein lysate.
Lane 3: ET1602-7 IP in GFP Tag fusion protein lysate.
Lane 4: ET1604-25 IP in GFP Tag fusion protein lysate.
Lane 5: ET1604-26 IP in GFP Tag fusion protein lysate.
Lane 6: ET1607-31 IP in GFP Tag fusion protein lysate.
Lane 7: R1312-2 IP in GFP Tag fusion protein lysate.

Blocking/Dilution buffer: 5% NFDM/TBST

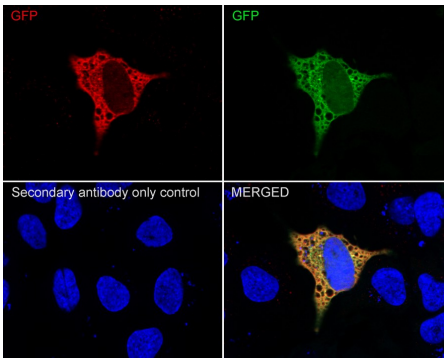

Fig3: Immunocytochemistry analysis of HeLa cells transfected with N-terminal GFP labeling GFP with Rabbit anti-GFP antibody (ET1607-31) at 1/500 dilution.

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-GFP antibody (ET1607-31) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) 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. Counterstained with GFP (green). Nuclear DNA was labelled in blue with DAPI.

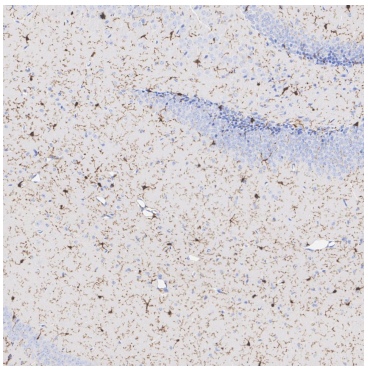

Fig4: Immunohistochemical analysis of paraffin-embedded mouse brain tissue transfected with GFP-tagged CX3CR1 with Rabbit anti-GFP antibody (ET1607-31) at 1/5,000 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1607-31) 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.

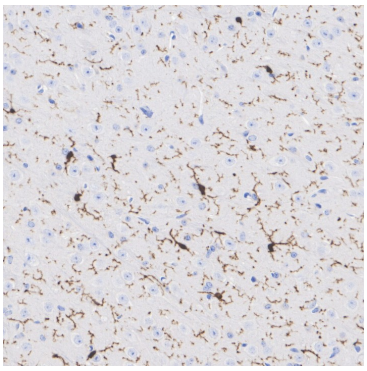

Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue transfected with GFP-tagged CX3CR1 with Rabbit anti-GFP antibody (ET1607-31) at 1/5,000 dilution.

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_2O and PBS, and then probed with the primary antibody (ET1607-31) 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.

Background References

1. Yu, H. et al. 2016. AAV-Mediated Gene Transfer to Dorsal Root Ganglion. Methods in molecular biology (Clifton, N.J.). 1382: 251-61.
2. Yamaoka, M. et al. 2016. PI3K regulates endocytosis after insulin secretion by mediating signaling crosstalk between Arf6 and Rab27a. J. Cell. Sci. 129: 637-49.