iFluor™ 594 Conjugated Goat anti-mouse IgG polyclonal Antibody_

HA1126

Product Type:	Goat polyclonal IgG, secondary antibodies
Species reactivity:	Mouse
Applications:	IF-Cell, IF-Tissue, FC, IHC-Fr
Description:	Whole IgG antibodies are isolated as intact molecules from antisera by immunoaffinity chromatography. They have an Fc portion and two antigen binding Fab portions joined together by disulfide bonds and therefore they are divalent. The average molecular weight is reported to be about 160 kDa. The whole IgG form of antibodies is suitable for the majority of immunodetection procedures and is the most cost effective. iFluor™ 594 dyes have fluorescence excitation and emission maxima of ~590 nm and ~610 nm respectively. iFluor™ 594 family is pH-independent from pH 3 to 11. These spectral characteristics make this new dye family an excellent alternative. iFluor™ 594 is much easier to be conjugated with RPE with much higher conjugation yield, and the resulted RPE-iFluor™ 594 tandem has better FRET efficiency. iFluor™ 594 SE is reasonably stable and shows good reactivity and selectivity with protein amino groups.
Conjugate:	iFluor™ 594, Ex: 588nm; Em: 604nm.
lmmunogen:	Mouse IgG (H+L).
Recommended Dilutions:	
IF-Cell	1:500-1:1,000
IF-Tissue	1:500-1:1,000
FC	1:500-1:1,000
IHC-Fr	1:500-1:1,000
Storage Buffer:	Preservative: 0.02% Sodium azide Constituents: 30% Glycerol, 1% BSA, 68.98% PBS
Storage Instruction:	Store at +4 $^\circ\!\!\mathbb{C}$ after thawing. Aliquot store at -20 $^\circ\!\!\mathbb{C}$. Avoid repeated freeze / thaw cycles.

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images



Fig1: Immunofluorescence analysis of paraffin-embedded rat cerebellum tissue labeling GFAP (EM140707) and NeuN (ET1602-12).

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies GFAP (EM140707, red) at 1/500 dilution and NeuN (ET1602-12, green) at 1/50 dilution at +4°C overnight, washed with PBS. Goat Anti-Mouse IgG H&L (iFluor TM 594, HA1126) and Goat Anti-Rabbit IgG H&L (iFluor TM 488, HA1121) were used as the secondary antibodies at 1/1,000 dilution. DAPI was used as nuclear counterstain.

Fig2: Immunocytochemistry analysis of HeLa cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) at 1/250 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-beta Actin antibody (HA722023) at 1/250 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluorTM 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-Goat Anti-Mouse IgG H&L (iFluorTM 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) at 1/100 dilution.



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Secondary antibody only control
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Fig4: Immunocytochemistry analysis of L6 cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) 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-beta Actin antibody (HA722023) at 1/100 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluorTM 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-Goat Anti-Mouse IgG H&L (iFluorTM 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.



Fig5: Flow cytometric analysis of A549 cells labeling Dynamin 1.

Cells were fixed and permeabilized.Then stained with the primary antibody (EM1901-44, 1ug/ml) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4 °C for an hour, the cells were stained with a iFluor TM 594 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1126) at 1/1,000 dilution for 30 minutes at +4 °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".

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