iFluor™ 488 Conjugated Goat anti-rabbit IgG polyclonal Antibody

HA1121

Product Type:	Goat polyclonal IgG, secondary antibodies
Species reactivity:	Rabbit
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. Although FITC is still the most popular fluorescent labeling dye for preparing green fluorescent bioconjugates, there are certain limitations with FITC, such as severe photobleaching for microscope imaging and pH-sensitive fluorescence. Protein conjugates prepared with iFluor™ 488 dyes are far superior compared to conjugates of fluorescein derivatives such as FITC. iFluor™ 488 conjugates are significantly brighter than fluorescein conjugates and are much more photostable. Additionally, the fluorescence of iFluor™ 488 is not affected by pH (4-10). This pH insensitivity is a major improvement over fluorescein, which emits its maximum fluorescence only at pH above 9. iFluor™ 488 SE dye is reasonably stable and shows good reactivity and selectivity with protein amino groups.

Conjugate: iFluor™ 488, Ex: 491nm; Em: 516nm.

Immunogen: Rabbit 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℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

Images

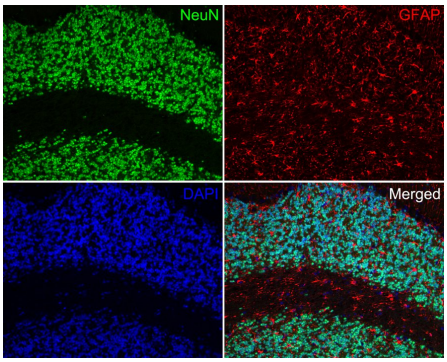

Fig1: Immunofluorescence analysis of paraffin-embedded rat cerebellum tissue labeling NeuN (ET1602-12) and GFAP (EM140707).
The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies NeuN (ET1602-12, green) at 1/50 dilution and GFAP (EM140707, red) at 1/500 dilution at +4℃ overnight, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) and Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) were used as the secondary antibodies at 1/1,000 dilution. DAPI was used as nuclear counterstain.

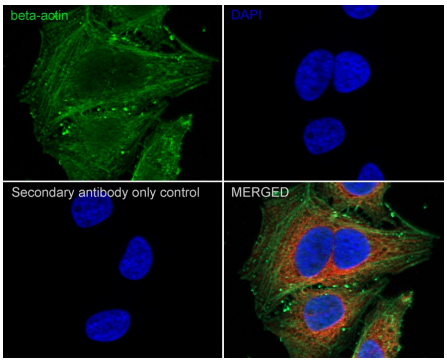

Fig2: Immunocytochemistry analysis of HeLa cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) at 1/250 dilution.

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 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

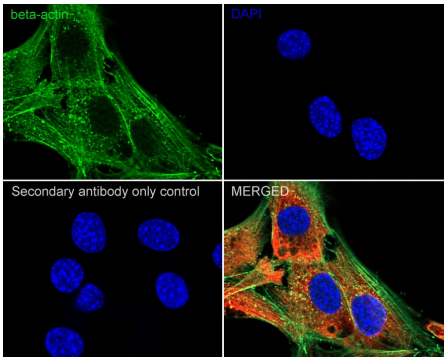

Fig3: Immunocytochemistry analysis of NIH/3T3 cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) at 1/100 dilution.

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 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

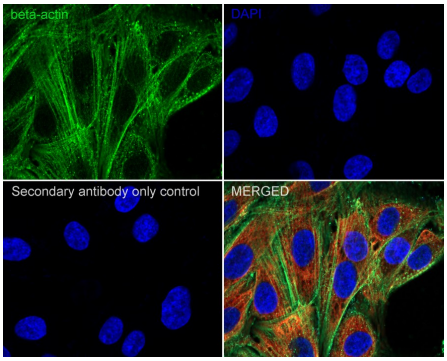

Fig4: Immunocytochemistry analysis of L6 cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) at 1/100 dilution.

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 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

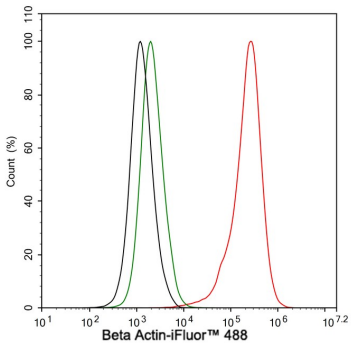

Fig5: Flow cytometric analysis of HeLa cells labeling beta Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722023, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

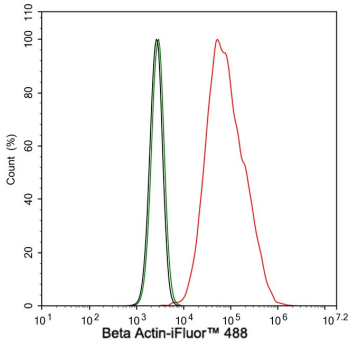

Fig6: Flow cytometric analysis of NIH/3T3 cells labeling beta Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722023, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

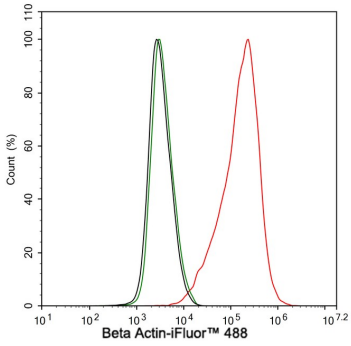

Fig7: Flow cytometric analysis of C6 cells labeling beta Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722023, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).