Donkey anti-Mouse IgG (H+L) Secondary Antibody, Qdot™ 625

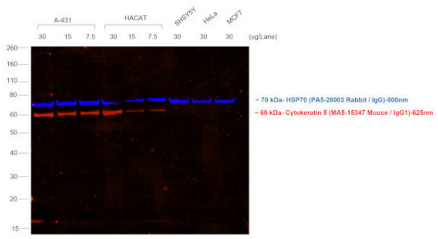
Product Details	
Size	100 µL
Species Reactivity	Mouse
Host/Isotype	Donkey / IgG
Class	Polyclonal
Type	Secondary Antibody
Conjugate	Qdot™ 625
Excitation/Emission Max	300/621 nm
Immunogen	Gamma Immunoglobins Heavy and Light chains
Form	Liquid
Concentration	1 µM
Purification	purified
Storage buffer	0.05M borate, pH 8.3, with 1M betaine
Contains	0.05% sodium azide
Storage conditions	4° C, store in dark
RRID	AB_2556492

Applications	Tested Dilution	Publications
Western Blot (WB)	1:50-1:500	-
Immunohistochemistry (IHC)	1:50	-
Immunocytochemistry (ICC/IF)	1:50-1:500	-
Flow Cytometry (Flow)	1:50	-

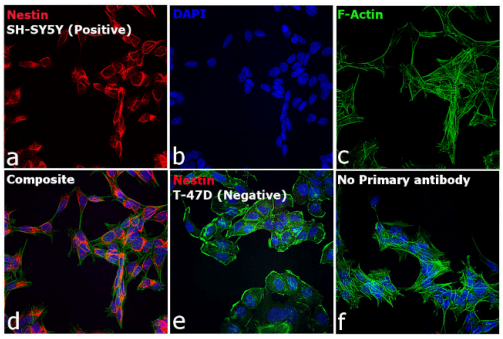
Product Specific Information

Qdot nanocrystals are composed of semi-conductor material to generate a fluorescent particle which is exceptionally bright and does not photobleach. Qdot nanocrystals paired with the correct optical filters are as much as 50 times brighter than traditional organic dyes.

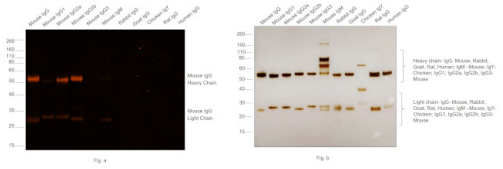
Product Images For Donkey anti-Mouse IgG (H+L) Secondary Antibody, Qdot™ 625



Mouse IgG (H+L) Secondary Antibody (Q22085) in WB
Multiplexed fluorescent western blot was performed using Donkey anti-Mouse IgG (H+L) Secondary Antibody, Qdot™ 625 (Product # Q22085). Whole cell extracts of A-431 (Lane 1, 2, 3), HaCaT (Lane 4, 5, 6), SH-SY5Y (Lane 7), HeLa (Lane 8) and MCF7 (Lane 9) were electrophoresed usingNuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were transferred onto anitrocellulose membrane (Product # IB23001) byiBlot® 2 Dry BlottingSystem (Product # IB21001). The blot was probed with Cytokeratin 5 Monoclonal Antibody (3E2F1) (Product # MA5-15347), and HSP70 Polyclonal Antibody (Product # PA5-28003). Secondary antibodies (Product # Q22085, 1: 500 dilution), and (Product # A32808, 1:20000 dilution) were used for detection of Cytokeratin 5, and HSP70 respectively. Fluorescent detection was performed usingiBrightFL1500 (Product # A44115). The anti-mouse secondary antibody (Product # R37115) specifically detects the mouse primary antibody.



Mouse IgG (H+L) Secondary Antibody (Q22085) in ICC/IF
Immunofluorescence analysis of Donkey anti-Mouse IgG (H+L) Secondary Antibody, Qdot™ 625 was performed using SH-SY5Y (positive model) and T-47D (negative model) cells stained with Nestin Monoclonal Antibody (10C2), eBioscience™ (Product # 14-9843-80). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, blocked with 2% BSA for 1 hour and labeled with 0.4 µg/mL primary antibody at 4 degree Celsius overnight at 4C. Donkey anti-Mouse IgG (H+L) Secondary Antibody, Qdot™ 625, 1:500 dilution) in 0.1% BSA in PBS for 45 minutes at room temperature, was used for detection of Nestin in the cytoskeleton (Panel a: Red). Nuclei (Panel b: blue) were stained with Hoechst33342 (Product # H1399). F-actin was stained with Alexa Fluor® 488 Phalloidin (Product # A12379, 1:500) (Panel c: green). Panel d represents the composite image. The specificity of the secondary antibody was proved by the absence of signal in T-47D (negative model for Nestin) due to no primary antibody binding (Panel e). Non-specific staining was not observed with secondary antibody alone (panel f). The images were captured at 40X magnification in CellInsight CX7 LZR High-Content Screening (HCS) Platform (Product # CX7A1110LZR) and externally deconvoluted (D.Sage et al./Methods 115 (2017) 28–41).



Mouse IgG (H+L) Secondary Antibody (Q22085) in WB
Western blot was performed using Donkey anti-Mouse IgG (H+L) Secondary Antibody, Qdot™ 625 (Product # Q22085) and ~55, 25 kDa bandscorresponding to Mouse IgG Heavy Chain and Light chain respectively were observed in Mouse IgG, Mouse IgG1, Mouse IgG2a, Mouse IgG2b, Mouse IgM but not in Rabbit IgG, Goat IgG, Chicken IgY, and Human IgG. Purified protein (500 ng) of Mouse IgG (Lane 1), Mouse IgG1 (Lane 2), Mouse IgG2a (Lane 3), Mouse IgG2b (Lane 4), Mouse IgG3 (Lane 5), Mouse IgM (Lane 6), Rabbit IgG(Lane 7), Goat IgG (Lane 8), Chicken IgY (Lane 9), Rat IgG (Lane 10), Human IgG (Lane 11) wereelectrophoresed usingNuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane(Product # IB23001) byiBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with Donkey anti-Mouse IgG (H+L) Secondary Antibody, Qdot™ 625 (Product # Q22085, 1:500 dilution) and detected using theiBrightFL1500 (Product # A44115). Silver staining was performed to establish equivalent loading of purified proteins using the Pierce™ Silver Stain Kit (Product # 24612) (Fig b). This antibody shows cross reactivity with Mouse IgM.

1 Reference

Cryo-electron tomography ofC. elegansmitochondria reveals how the ATP synthase dimer interface shapes crista membranes bioRxiv (2023)