An immunofluorescent staining review for Rab5a antibody (NB120-13253)

Hepatocytes grown on coverslips were briefly washed in PBS and fixed in 2% paraformaldehyde at room temperature for 15min and stained as previously described (Eum, Vallabhaneni et al. 2011). Hepatocytes were incubated with 2% bovine serum albumin (BSA) in PBS for 1 hr followed by five washes with PBS+0.5% BSA (PBB). The samples were then incubated with the antibodies diluted in PBB against Rab5a (1:200 dilution). Samples were washed five times with PBB followed by incubation in the appropriate Alexa Fluor 488 (1:500; Invitrogen) secondary antibodies diluted in PBB. Samples were washed with PBS before placing a coverslip using Gelvatol (23 g of poly(vinyl alcohol 2000), 50 ml of glycerol, 0.1% sodium azide to 100 ml of PBS). Positively-stained cells in six random fields were imaged on a FluoView 1000 confocal scanning microscope (Olympus, Center Valley, PA). Imaging conditions were maintained at identical settings within each antibody labeling experiment with original gating performed using the negative control.