Avidin Agarose

Precipitation

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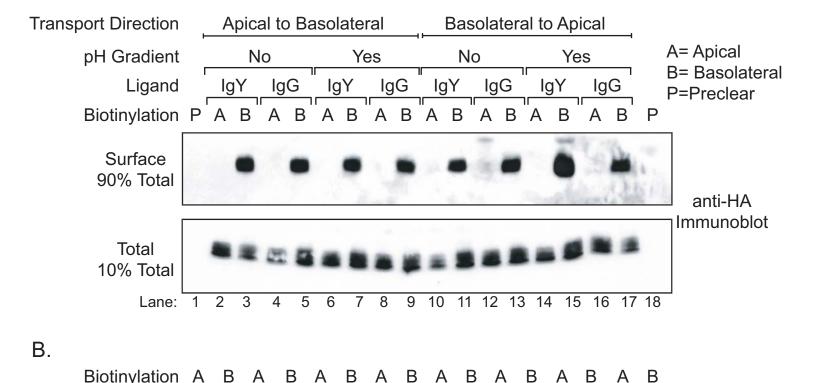
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Supplemental Figure 1. The addition of human IgG to either the apical or basolateral compartments, with or without a pH gradient, does not result in hFcRn redistribution to the apical plasma membrane in MDCK cells. Human IgG or chicken IgY were added to the apical or basolateral compartments of confluent monolayers to a final concentration of 100 µg/ml in the absence or presence of an additional pH gradient (pH 7.3 input, pH 7.3 output, or pH 6.0 input, pH 7.3 output, respectively). Following incubation at 37°C, 5% CO₂ for 12 hours, the apical or basolateral plasma membrane domains were selectively biotinylated as previously described. (A) FcRn immunoprecipitation, avidinagarose reprecipitation, and anti-HA immunoblotting were performed as described in Figure 5. (B) Avidin-agarose precipitation of the FcRn immunoprecipitation flow-through and anti-GP135 immunoblotting were performed as described in Figure 5. These data were confirmed utilizing two independent hFcRn+/h β_2 m+ MDCK clones. Data are presented from a single clone (n=3).

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anti-GP135

Immunoblot