





**Detection of human Phospho RPS6 (S235/S236) by western blot.** *Samples:* Whole cell lysate (35  $\mu$ g) from HeLa cells. Treatments consisted of growth in serum containing media (lane 1) or cultured for 24 hours in serum free media (lanes 2 to 5). At 24h, serum was replaced for 1 h (lanes 2 and 3) or serum starvation was continued (lanes 4 and 5) without rapamycin (lanes 3 and 5) with 50nM Rapamycin (lanes 2 and 4). *Antibodies:* Affinity purified rabbit anti-phospho RPS6 (S235/S236) antibody (Cat. No. A300-584A) used at 0.04  $\mu$ g/ml (upper panel). The blot was stripped and re-blotted for total RPS6 using affinity purified rabbit anti-RPS6 antibody (Cat. No. A300-557A) at 0.04  $\mu$ g/ml. *Detection:* Chemiluminescence with exposure times of 10 seconds (upper panel) or 30 seconds (lower panel).