Immunoprecipitations from eosinophils

Eosinophils obtained from CD2-IL-5Tg mice are activated with ligand in 96-well plates (37°C, 5%CO_2). The reaction is stopped at the indicated time points by adding M-PER lysis buffer (Pierce, Rockford, IL) supplemented with protease inhibitor cocktail (Sigma, St. Louis, MO) on ice for 30 min. The cell lysate was transferred to an eppendorf tube, and preclearing was performed using protein A/G beads (Pierce, Rockford, IL). Antibody is added to the precleared lysate (8 μg/ml, 1 h, 4°C, rotation) followed by protein A/G (1 h, 4°C, rotation). The immunoprecipitated complex is eluted from the protein A/G beads using Ig-elution buffer (Pierce, Rockford, IL).