Kinase assays

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Introduction

This protocol was used in the above *Nature Immunology* paper.

Procedure

1. Stimulate 10-20 x 10^6 OT-1 hybridoma T cells by incubating 5ug/ml/5 X 10^7 cells of anti-CD3 (2C11, ATCC CRL-1975) and 20µg anti-CD28 (37.51) on ice for 30 minutes. Crosslink with 50µg goat-anti-hamster antibody (MP Biomedicals 55394) at 37^{o}C for 30 minutes (keeping cells at 5 X 10^7/ml).

2.Lyse cells in TNE (50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 µg/ml aprotinin, 1 µg/ml leupeptin, 1 mM PMSF, and 1-5 mM Na_3VO_4. Clear lysates by centrifugation at 12,000 X g for 10 minutes at 4^{o}C.

3. Set up immunopreciptations of Dlgh1 using 40 µl of protein G (50% slurry so 20µl bead volume) (GE Healthcare Bio-Sciences 17-0618-02) and 10 µl (2.5µg) of Dlgh1 (BD Transduction Lab 610875) or p38 (2.0µg) (Santa Cruz, C-20, sc-535) antibody. Tumble at 4uC for 1-2 hours. Save some of this supernatant to check efficiency of IP.
4. Wash once with 500µl of kinase buffer (25mM Tris pH 7.5, 5mM B-glycerophosphate, 2mM DTT, 0.1 mM Na_3VO_4 and 10mM $MgCL_2$; Cell signaling 9802)
5. In a final volume of 50µl have 1µg of GST-ATF2 (Cell signaling, 9224S) and/or 50µM unlabeled ATP (Cell signaling, 9804) with immunopreciptates in kinase buffer. (20µl of beads, 1µl of ATP, 29 µl of kinase buffer) MAKE SURE TO DO ALL OF THIS ON ICE
6. Incubate at 30^{o}C for 5-20 minutes.
7. Add 20µl 5X Loading buffer to stop reaction and boil for 5 minutes

Scaffold protein Dlgh1 coordinates alternative p38 kinase activation, directing T cell receptor signals toward NFAT but not NFkappa-B transcription factors

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