Kinase assays

June Round

Method Article

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Abstract

Introduction

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

Procedure

1. Stimulate $10\text{--}20 \times 10^6$ OT-1 hybridoma T cells by incubating $5\mu\text{g/ml}$ / 5×10^7 cells of anti-CD3 (2C11, ATCC CRL-1975) and $20\mu\text{g}$ anti-CD28 (37.51) on ice for 30 minutes. Crosslink with $50\mu\text{g}$ goat-anti-hamster antibody (MP Biomedicals 55394) at 37°C for 30 minutes (keeping cells at $5 \times 10^7/\text{ml}$). 2. Lyse cells in TNE (50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, $1\mu\text{g/ml}$ aprotinin, $1\mu\text{g/ml}$ leupeptin, 1 mM PMSF, and 1–5 mM Na_3VO_4). Clear lysates by centrifugation at $12,000 \times g$ for 10 minutes at 4°C . 3. Set up immunoprecipitations of Dlg1 using $40\mu\text{l}$ of protein G (50% slurry so $20\mu\text{l}$ bead volume) (GE Healthcare Bio-Sciences 17-0618-02) and $10\mu\text{l}$ ($2.5\mu\text{g}$) of Dlg1 (BD Transduction Lab 610875) or p38 ($2.0\mu\text{g}$) (Santa Cruz, C-20, sc-535) antibody. Tumble at 4°C for 1–2 hours. Save some of this supernatant to check efficiency of IP. 4. Wash once with $500\mu\text{l}$ 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 $50\mu\text{l}$ have $1\mu\text{g}$ of GST-ATF2 (Cell signaling, 9224S) and/or $50\mu\text{M}$ unlabeled ATP (Cell signaling, 9804) with immunoprecipitates in kinase buffer. ($20\mu\text{l}$ of beads, $1\mu\text{l}$ of ATP, $29\mu\text{l}$ of kinase buffer) MAKE SURE TO DO ALL OF THIS ON ICE 6. Incubate at 30°C for 5–20 minutes. 7. Add $20\mu\text{l}$ 5X Loading buffer to stop reaction and boil for 5 minutes