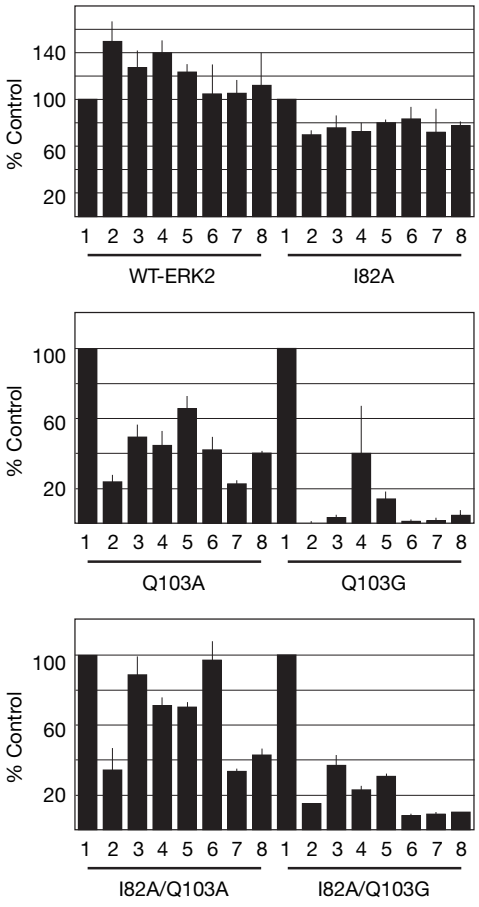

FIGURE 3. Analog inhibition assay. FLAG-ERK2 wild-type and various mutant proteins were immunoprecipitated from EGF-stimulated COS-1 cells and used in in vitro kinase assays with 20 μg of myelin basic protein (MBP) as substrate. Reactions also contained 10 μM ATP, 10 μCi/reaction [γ-^{32}P]ATP, and either distilled water (1) or 100 μM ATP analog. The ATP analogs used were: N-6-benzyl ATP (2); N-6-cyclopentyl ATP (3); N-6-(p-methyl)benzyl ATP (4); N-6-(1,3-dimethyl)butyl ATP (5); N-6-(3,3-dimethyl)butyl ATP (6); N-6-(2-phenethyl) ATP (7); N-6-(1-methyl)butyl ATP (8). MBP phosphorylation was quantitated by Cerenkov counting of the MBP bands excised from Ponceau-S-stained membranes. The values were normalized to a reaction without ATP analog (lane *1*), which was set at 100%. (Reprinted, with permission, from Eblen et al. 2003.)

Protein–Protein Interactions: A Molecular Cloning *Manual*, 2nd Ed., © 2005 by Cold Spring Harbor Laboratory Press, Chapter 24, Figure 3.