

QA/QC:

Pim1 protein (100 ng/μl concentration) was diluted to 20 ng/μl with assay dilution buffer (4 mM MOPS + 2.5 mM β-glycerophosphate + 1 mM EGTA + 0.4 mM EDTA + 4 mM MgCl₂ + 0.05 mM DTT; pH 7.2), followed by 2-fold serial dilutions, and then the 10 μl diluted proteins were used to phosphorylate the S6K substrate peptide (CKRRRLASLR) in the following assay condition:

- 10 μl diluted Pim1 protein
- 10 μl S6K substrate peptide (1 mg/ml stock)
- 5 μl [³²P] ATP mixture (250 μM ATP, 0.16 μCi/μl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 μl of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.

Fig. 1 Pim1 activity assay

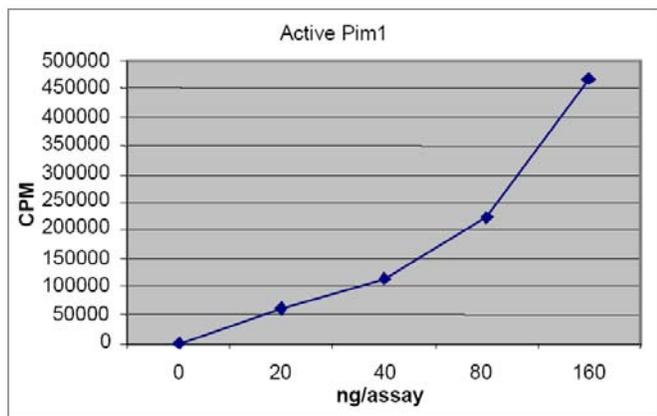
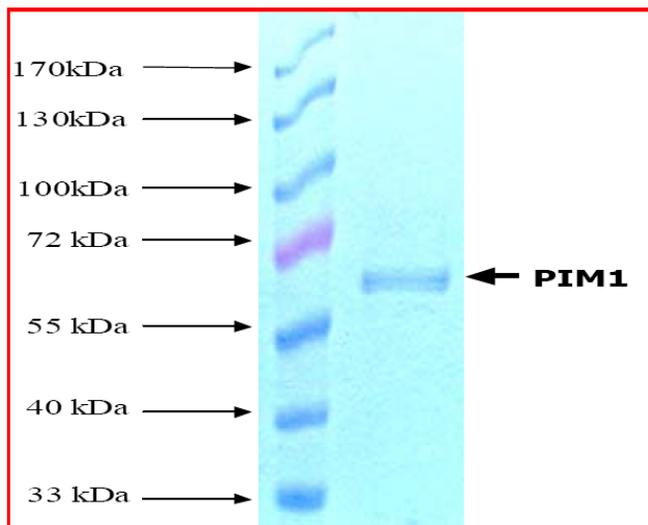


Fig. 2 Pim1 protein gel



Please note: always centrifuge vials before opening.

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