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Rab7 Phosphorylation reaction

In 1 collection

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ABSTRACT

Phosphorylation of Rab7 at S72 using TBK1

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Phosphorylation reaction

1 Mix phosphorylation reaction mixture. Combine 4 uM human Rab7 with 400 nM purified human strep-tagged TBK1 in a buffer consisting of 50 mM HEPES 7.5, 150 mM NaCl, 10 mM MgCl2, and 200 uM ATP. Incubate at room temperature for 3 hours.

2 Pass reaction mixture over a strep-tactin sepharose gravity column. Pass reaction mixture over resin x4 in order to remove all strep tagged TBK1 from solution.

3 Buffer exchange via dilution and centrifugation or dialysis overnight in order to recover purified Rab7

PhosTag gel

4 In order to assess degree of phosphorylation, prepare a 15 ug sample of phosphorylated Rab7 for PhosTag gel electrophoresis. Buffer exchange the protein into pure water via dilution and concentration with centrifugal concentrator.

As a positive control, prepare a sample of purified Casein, purchasable in bulk as a lyophilized solid. This protein is natively phosphorylated. Incubate with lambda phosphatase in order to produce its unphosphorylated form. Run the native casein as a positive control for the PhosTag gel, and the unphosphorylated form as a negative control.

5 Follow guidelines for Fujifilm Wako's "SuperSep" precast PhosTag gel. Add loading buffer to 1x, and run gel at ~120 V until the dye front reaches the bottom of the gel.

6 Stain gel via Coomassie staining, and image.