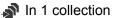


MAR 07, 2024

Rab7 Phosphorylation reaction



Dan Tudorica¹

¹University of California, Berkeley



Dan Tudorica Hurley Lab, QB3, UC Berkeley

ABSTRACT

Phosphorylation of Rab7 at S72 using TBK1





DOI:

dx.doi.org/10.17504/protocols.io. 261ged92wv47/v1

Protocol Citation: Dan Tudorica 2024. Rab7 Phosphorylation reaction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ged92wv47/v1

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Protocol status: Working

Created: Nov 09, 2023

Last Modified: Mar 07, 2024

Oct 7 2024

PROTOCOL integer ID: 90730

Phosphorylation reaction

- 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

- 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.
- 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.



Oct 7 2024