V. 1.0

p50Rho GAP protein
(human recombinant)
Cat. # GAP01

Upon arrival store at 4°C (desiccated)
See datasheet for storage after reconstitution

Material

Human p50RhoGAP protein (sometimes called Cdc42-GAP or Rho GAP) is a GTPase activating protein that catalyses the hydrolysis of GTP for Rac, Rho and Cdc42 proteins. The protein is supplied as a GST fusion protein which has been produced by overexpression in a bacterial expression system and purified by glutathione affinity purification. The protein is 78 kD in size and consists of the 50 kD full length p50 RhoGAP1 and an amino terminal GST protein tag (approx. 28 kD, runs at 32kD on an SDS gel). The protein is supplied as a lyophilized powder. Each tube contains 50 µg of protein.

Storage and Reconstitution

The protein should be reconstituted to 1 mg/ml by the addition of 50 ul of distilled water. The protein will be in the following buffer; 2 mM Tris pH 7.5, 0.5 mM $MgCl_2$, 0.5% sucrose, 0.1% dextran. In order to maintain high biological activity of the protein, it is strongly recommended that the protein solution be supplemented with DTT to 1 mM final concentration, aliquoted into "experiment sized" aliquots and snap frozen in liquid nitrogen. The reconstituted protein can be stored a -70°C for up to 6 months. The protein must not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C or -70°C for 1 year when stored dessiccated (<10% humidity).

Purity

Protein purity is determined by scanning densitometry of Coomassie blue stained protein on a 4-20% polyacrylamide gradient gel. The protein was determined to be >70% pure. (see Figure 1). The major contaminant running at 32 kD is GST and does not interfere with the activity of p50 GAP.

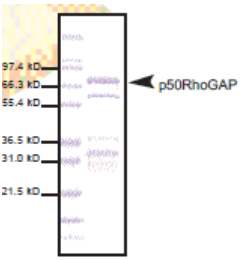


Figure1. p50RhoGAP Protein Purity Determination. A 10 ug sample of recombinant GAP01 protein (molecular weight approx. 78 kD) was separated by electrophoresis in a 4-20% SDS-PAGE system. The protein was stained with coomassie blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat.# ADV02). Mark 12 molecular weight markers are from Invitrogen.

Biological Activity Assay

Biological activity of p50 RhoGAP protein is determined by the ability of the protein to catalyse GTP hydrolysis by RhoA and Rac1 proteins.

Reagents

1. Recombinant p50 RhoGAP (Cat.# GAP01)
2. Recombinant RhoA-His protein (Cat.# RH01)
3. Recombinant Rac1-His protein (Cat.# RC01)
4. GTP stock (100 mM) (Cat.# BST06-001)
5. 2X reaction buffer (80 mM PIPES pH 7.5, 20 mM EDTA, 20 mM NaCl)
6. CytoPhos reagent (Cat.# BK054)

All reagents (except GAP01) are available in biochemical kit Cat # BK105

Equipment

1. Spectrophotometer set to 650 nm
2. Corning 96-well half area plates (Cat # 3686).

Method

1. Resuspend all protein reagents in distilled water to give 1 mg/ml final concentration.
2. Add 15 µl of 2X reaction buffer to five individual wells of a 96 well plate.
3. Supplement the wells with the following reagents ; well # 1 = 5 µl Rac1 protein, well # 2 = 5 µl Rac1 protein plus 10 µl GAP01, well # 3 = 5 ul RhoA protein, well # 4 = 5 µl RhoA protein plus 10 µl GAP01, well # 5 = 10 µl GAP01. Make up the volume of each reaction to 30 µl using distilled water.
4. Initiate all reactions by adding GTP to 200 µM final concentration and incubate at 37°C for 20 minutes.
5. Stop the reaction by adding 120 µl of CytoPhos reagent into each well.
6. Allow the green color to develop for 10 minutes.
7. Read reactions at 650 nm. An example of standard results are given in Figure 2, increased OD650 readings indicate enhanced GTP hydrolysis in those samples.

NOTE: This is a qualitative analysis of GAP activity. Specific GAP activity in nmoles/min/mg of small G-protein can be determined by taking timepoints from a scaled up GAP reaction. Readings can be quantitated against a standard phosphate curve. The standard curve can be performed as follows;

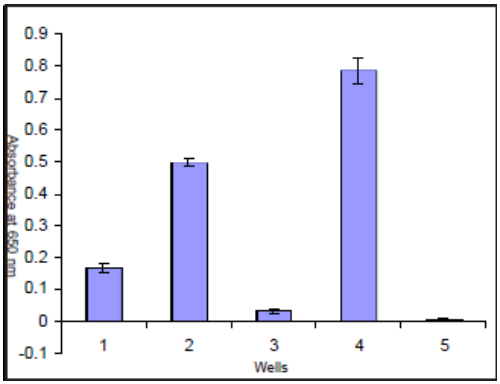
a. Prepare a 0.1 mM KH2PO4 solution in distilled water and add 1,2,5 and 20 µl to individual wells of a half area plate.
b. Bring the volumes to 30 µl with distilled water and add 120

Phone: (303) 322.2254 **Fax:** (303) 322.2257
Customer Service: cserve@cytoskeleton.com
Technical Support: tservice@cytoskeleton.com

µl of CytoPhos reagent into each well.

c. Proceed with color development as described above.

Figure 2.
Rac1 & RhoA GAP Assay



Rac1 (wells 1 & 2) and RhoA (wells 3 & 4) proteins were incubated at 37°C in the absence (wells 1 & 3) or presence (wells 2 & 4) of p50RhoGAP catalytic domain protein. Each 30 µl reaction contains 5 µg of either Rac1 or RhoA protein and +/- 10 µg of p50RhoGAP protein. Well 5 contains p50RhoGAP protein only. All reactions were incubated at 37°C for 20 minutes followed by the addition of 120 µl of CytoPhos reagent. After a 10 minute incubation the reactions were read at 650nm. Increased OD650 indicates enhanced GTP hydrolysis.

Product Uses

- Study of GAP activity with different GTPases.
- Identification of GAP binding proteins
- Postive control for other RhoGAP studies.

References

1. J. Biol. Chem. (1994) 269: 1137-1142.

Product Citations/Related Products

For the latest citations and related products please visit www.cytoskeleton.com.