V. 2.0

RhoA His protein: Constitutively Active
(human recombinant)
Cat. # R6301

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

Material

The constitutively active form of the human RhoA protein has been produced in a bacterial expression system. This protein has a glutamine to leucine substitution at amino acid 63, creating a constitutively active mutant protein that will not hydrolyze GTP. The mutant protein is also a very poor substrate for nucleotide exchange (see Biological Activity Assay). The recombinant protein contains a run of 6 histidine residues (His-tag) at its amino terminus. The estimated molecular weight of RhoA L63-His is 22 kDa. The protein is supplied as 10 ug of lyophilized powder.

Storage and Reconstitution

The protein should be reconstituted to 1 mg/ml by the addition of 10 ul of distilled water. The protein will be in the following buffer: 2 mM Tris pH 7.6, 0.5 mM $MgCl_2$, 0.5% sucrose, and 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 protein can be stored at -70°C for 6 months. The protein must not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C for at least 1 year.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gradient gel. RhoA L63-His protein was determined to be >90% pure (see Figure 1).

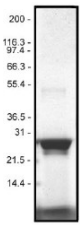

Figure 1. RhoA Constitutively Active His-tagged Protein Purity Determination. A 10 ug sample of recombinant RhoA L63 -His tagged protein was separated by electrophoresis in a 4-20% SDS-PAGE system. The RhoA-His protein migrates slightly higher than its predicted molecular weight of 22 kDa. The protein was stained with Coomassie Blue. Protein quantitation was preformed using the Precision Red Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

A standard biological assay for R6301 consists of a pull-down assay using GST-tagged Rhotekin RBD beads (Cat.# RT02). The Rho Binding Domain (RBD) of the Rho effector protein Rhotekin has a high affinity for GTP-bound Rho. Using this assay, the amount of biologically active GTP-bound RhoA or constitutively active mutant RhoA can be determined. Stringent quality control ensures that >80% of the RhoA protein is pulled down in this assay.

Reagents

1. GST-tagged Rhotekin RBD protein on beads (Cat.# RT02)
2. Recombinant RhoA L63-His protein (Cat.# R6301)
3. Loading buffer (150 mM EDTA)
4. Stop buffer (600 mM $MgCl_2$)
5. Wash buffer (25 mM Tris pH 7.5, 30 mM $MgCl_2$, 40 mM NaCl)
6. Lysis buffer (10 mM Tris pH 7.5, 1 mM DTT, 25 mM NaCl)
7. GTPγS (20 mM solution) (Cat.# BS01)
8. GDP (100 mM solution)
9. BSA (10 mg/ml)
10. Anti-RhoA monoclonal antibody (Cat.# ARH03)

Equipment

1. Microfuge at 4°C
2. SDS-PAGE and Western blot apparatus

Method

1. RhoA L63-His was resuspended to 0.1 ug/ul by the addition of 100 ul ice cold lysis buffer.
2. Add 200 ng RhoA L63-His to 23 ul Lysis buffer and 2 mM GTPγS on ice.
3. Add 200 ng RhoA L63-His to 23 ul Lysis buffer and 10 mM GDP on ice.
4. Add $1/10^{th}$ the volume of loading buffer to each tube (final conc. 10 mM), and incubate at room temp. for 15 min.
5. Stop the reaction by adding $1/10^{th}$ the volume of stop buffer to each tube (final conc. 60 mM)
6. Resuspend the RT02 Rhotekin beads to 0.83 mg/ml by the addition of 600 ul of cold water.
7. To each reaction tube add 215 ul wash buffer, 60 ul (50 ug) RT02 beads, and 20 ul 10 mg/ml BSA.
8. Gently rotate the tubes at 4°C for 1 hr.
9. Centrifuge out the beads at 6800 x g in a microfuge at 4°C for 1 min.
10. Remove the supernatant and wash the beads in 1 ml of wash buffer. Remove the beads to a new tube.
11. Centrifuge out the beads and resuspend the beads from each tube in 25 ul of SDS sample buffer.

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12. The bead and supernatant samples can now be analyzed by Western blot procedure using a RhoA specific monoclonal antibody (Cat.# ARH03).
13. Typical assay results are shown in Figure 2.

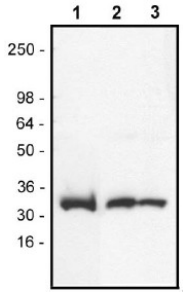

Figure 2. Binding of Rhotekin RBD GST Tagged Protein Beads to Constitutively Active Form of RhoA *In Vitro*.
RhoA L63-His was incubated with either GTPγS (lane 2), or GDP (lane 3) as described in the method. The proteins were then incubated with 50 ug of Rhotekin RBD GST tagged beads, separated by centrifugation, and subjected to Western blot analysis using a RhoA specific monoclonal antibody. Lane 1 shows 200 ng of recombinant RhoA-His control protein. In this same biological assay only GTP bound wild-type RhoA is detected (data not shown). See Blue molecular weight markers are from Invitrogen.

Product Uses

- Identification of active RhoA binding proteins
- Study of RhoA function *in vivo* by the introduction of RhoA L63-His into live cells

Product Citations/Related Products
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