V. 1.0

C3 Transferase Protein
(Bacterial recombinant)
Cat. # CT03

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

Material

The exoenzyme C3 transferase from Clostridium botulinum has been produced in a bacterial expression system. The recombinant protein contains six histidine residues at its amino terminus (His tag), and has a molecular weight of approximately 24 kDa. C3 transferase is an ADP-ribosyl transferase that selectively ribosylates Rho proteins in the effector-binding domain on asparagine 41, rendering them inactive. C3 transferase has low affinity for other members of the Rho family such as Cdc42 and Rac1. C3 transferase protein is supplied as a white lyophilized powder.

Storage and Reconstitution

Briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 1 mg/ml by the addition of 25 ul of distilled water. The protein will be in the following buffer; 500 mM Imidazole pH 7.5, 50 mM Tris HCl pH 7.5, 1.0 mM $MgCl_2$, 200 mM NaCl, 5% sucrose and 1% dextran. In order to maintain high biological activity of the protein, it is recommended that the protein solution be supplemented with DTT to 1 mM, aliquoted into "experiment sized" amounts, snap frozen in liquid nitrogen and stored at -70°C. The protein is stable for 6 months if stored at -70° C. The protein should not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for 1 year.

Purity

Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 12% polyacrylamide gel. C3 transferase protein was determined to be 80% pure (see Figure 1).

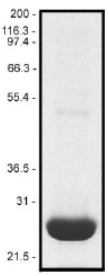

Figure 1. C3 transferase Protein Purity Determination. A 20 ug sample of recombinant C3 transferase protein (molecular weight approx. 24 kDa) was separated by electrophoresis in a 12% SDS-PAGE system, and stained with Coomassie Blue. Protein quantitation was performed using the Precision Red Protein Assay Reagent (Cat.# ADV02). Mark12 molecular weight markers are from Invitrogen.

Biological Activity Assay

The biological activity of recombinant C3 transferase can be determined from its ability to ADP ribosylate native Rho in human platelet extracts. A standard biological assay for monitoring the ADP ribosylation of Rho consists of an *in vivo* ribosylation reaction followed by non-denaturing gel electophoresis and Western blot analysis (1). Stringent quality control ensures that > 80% of native Rho protein is ADP ribosylated by the recombinant C3 transferase.

Reagents

1. Recombinant C3 transferase (25 ug, Cat. # CT03).
2. Human platelet extract (20 mg/ml) prepared in 50 mM PIPES pH 7.0,130 mM NaCl, 1 mM PMSF, 1 mM DTT, 5 ug/ml leupeptin, 0.5% Triton X-100.
3. Ribosylation buffer (5x): 250 mM PIPES pH 7.0, 10 mM $MgCl_2$, 5 mM EDTA, 0.05% SDS.
4. 10 mM β-NAD (Sigma Cat. # N-704).
5. 100 mM Thymidine (Sigma, Cat. # T-9250).
6. 1 M DTT
7. 5x Tris-Glycine native sample buffer: 10 mM Tris-HCl pH 8.0, 100 uM $MgCl_2$, 10 uM GDP, 0.5 mM DTT, 50% glycerol, 1.0% (w/v) bromophenol blue.
8. Protease inhibitor cocktail, 100x (Cat. # PIC02).
9. Tris-Glycine running buffer: 25 mM Tris-base pH 8.3, 192 mM glycine.
10. Non-denaturing 8% polyacrylamide Tris-Glycine gel (Novex gels, Invitrogen).
11. Tris-Glycine transfer buffer: 25 mM Tris-base pH 8.3, 192 mM glycine. No methanol.
12. Anti-Rho monoclonal antibody (Cat. # ARH01)

Equipment

1. Water bath at 37°C
2. Electrophoresis and Western blot apparatus at 4°C

Method

1. Dilute C3-transferase (Cat. # CT03) to 0.5 ug/ul in 50 mM PIPES pH 7.0
2. Prepare reaction buffer by adding the following components to 100 ul of 5x ribosylation buffer: 1 ul of 1 M DTT, 5 ul of 100x protease inhibitor cocktail, 2 ul of 10 mM β−NAD, and 10 ul of 100 mM thymidine.
3. Add 5 ul (100 ug) of platelet extract to microcentrifuge tubes on ice.
4. Add 4 ul of the supplemented reaction buffer to each tube on ice.
5. Add 2 ul (1 ug) of C3-transferase (Cat. # CT03) to one tube (experiment) and 2 ul of reaction buffer to a second tube (control).

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

6. Bring the volume of each tube to 20 ul with Milli-Q water and incubate at 37°C for 30 min. Final concentrations of the reaction components is shown in Table 1.
7.

Table 1. ADP ribosylation reaction components

Reaction buffer	1x
DTT	2 mM
NAD	40 uM
Thymidine	2 mM
Extract	100 ug
C3 Transferase	1 ug

7. Add 5 ul of native sample buffer to each tube to stop the reactions.
8. Electrophorese the samples at 4°C on an 8% non-denaturing gel at 80 V for 4-5 h.
9. Transfer the proteins to PVDF membrane at 4°C at 100 V for 90 min.
10. The membrane can now be probed with a Rho specific monoclonal antibody (Cat. # ARH01)
11. Typical assay results are shown in Figure 2.

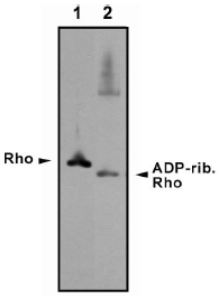

Figure 2. ADP-ribosylation of Rho Protein in Human Platelet Extracts. Human platelet extract (100 ug) was ADP-ribosylated with C3 transferase (1 ug) for 30 min at 37°C as described in the method The extract proteins were then separated by non-denaturing gel electrophoresis and processed for Western blot analysis use a Rho monoclonal antibody. ADP- ribosylated Rho (lane 2) shows increased migration in the gel compared to non-modified Rho (lane 1).

Product Uses

- Inhibition of Rho activity *in vivo* by microinjection or pinocytic uptake into cells.
- Inhibition of Rho activity *in vitro*.

References

1. Aktories K. and Just I. 1995. *Methods Enzymol.* 256:184-195.

Product Citations/Related Products

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