V. 1.4

ARNO GEF Protein: Sec7 domain (αα31- 267) wild type.
(Human recombinant, 6xHis tagged)
Cat. # GE07
Lot: 003   Amount 1 x 100 µg
Upon arrival store at 4°C (desiccated)
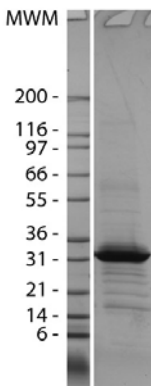**See datasheet for storage after reconstitution**

Background

ARNO is a guanine exchange factor for Arf family proteins (1). It's intracellular function is to regulate Arf proteins by transducing the upstream signal from phospholipids, kinases and Arf proteins themselves (2). Arno functions in large concerted cellular events that require re-organization of membranes e.g. cell migration (3,4), phagocytosis (5), macropinocytosis (6), and insulin controlled organism level growth (7).

Material

The GEF domain (Sec7 domain) of human ARNO protein has been produced in a bacterial expression system. It contains six histidine residues at its amino terminus (His-tag). The accession number is X99753.1(embl). The molecular weight of ARNO-His GEF domain is approximately 33 kDa. The ARNO protein is supplied as a white lyophilized powder. Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gradient gel. ARNO protein was determined to be 90% pure. (see Figure 1).

Figure 1. GE07 Purity Determination. A 20 µg sample of recombinant GE07 (molecular weight approx. 33 kDa) was separated by electrophoresis in a 4-20% SDS-PAGE system and stained with Coomassie Blue. Protein quantitation was determined using the Precision Red Protein Assay Reagent (Cat. # ADV02).   Mark12 molecular weight markers are from Life Technologies Inc.

MWM: 200, 116, 97, 66, 55, 36, 31, 21, 14, 6

Storage and Reconstitution

Before reconstitution, briefly centrifuge to collect the product at the bottom of the tube. The protein should be reconstituted to 5 mg/ml with Milli-Q water (10 µl water per 50 µg protein).   When reconstituted, the protein will be in the following buffer: 50 mM Tris pH 7.6, 20 mM NaCl, 1 mM $MgCl_2$, 5% (w/v) sucrose and 1% (w/v) 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" amounts, snap frozen in liquid nitrogen and stored at -70°C. The protein is stable for six months if stored at -70°C. The protein must not be exposed to repeated freeze-thaw cycles. The lyophilized protein is stable at 4°C desiccated (<10% humidity) for one year.

Biological Activity Assay

The biological activity of GE07 can be determined from its ability to catalyze nucleotide exchange on Arf proteins using the nucleotide exchange assay of GDP for mant-GTP. The exchange reactions can be performed by adding EDTA or Arf GEF h.s. ARNO protein to a reaction hence providing a control condition for exchange (see kit Cat. # BK100). The reaction is monitored by fluorescence measurement at 360nm Ex / 440nm Em. Stringent quality control ensures that the exchange rate of mant-GTP is enhanced two fold in the presence of 1uM ARNO. Exchange rates are similar to published examples of ARNO catalyzed exchange in >1 mM Mg2+ containing buffer (Bourgoin et al. 2002, see Figure 2) and with <1 µM Mg2+ containing buffer (Beraud-Defour et al. 1998).

Reagents

1.   ARNO protein (Cat. # GE07)
2.   Arf1(17aa del) protein (Cat. # FR02)
3.   2x Exchange buffer (40 mM Tris pH 7.5, 100 mM NaCl, 20 mM $MgCl_2$, 2 mM DTT, 100 µg/ml BSA, and 1.5 µM Bodipy -GTP or mant-GTP).

Exchange Buffer is available in the RhoGEF Exchange Assay Biochem kit (Cat # BK100).

Method

1.   Dilute ARNO protein (Cat# GE07) to 0.6 µg/µl (15 µM).

2.   Dilute Arf1 (Cat# FR02) to 1.25 µg/µl (50 µM).

3.   Dissolve exchange buffer in 5 ml nanopure water and keep in room temperature.

4. Add the following components together and mix well by pipetting or gentle vortex:

Component	per well
2x Exchange Buffer	50 µl
50 µM Arf1	10 µl
H_2O	36 µl

5. Aliquot to the assigned well and place the plate in the fluorimeter. Set up the fluorimeter (Excitation wavelength at 360 nm and emission wavelength at 440 nm) and start the reading.

6. After 5 cycles (150 seconds) pipette 10 µl ARNO (15 µM) protein or Dilution Buffer in respective wells and immediately pipette up and down twice and resume reading for at least 30 minutes.

7. Save the readings after the kinetic protocols are finished. The exchange rate can be calculated by reducing the data to Vmax with the software that accompanies the plate reader. The exchange curve can be achieved by export to Microsoft Excel.

Figure 2. ARNO catalyzed mant-GTP exchange assay.

Legend: Arf1(with 17 aa deletion, Cat. # FR02) (5 µM) was mixed with 1.25 µM GE07 or nanopure water (control) and pipetted into wells of a black 384-well low volume plate. At time zero, one volume of 2x concentrated Exchange Buffer was pipetted in to the wells and the reactions were monitored for 30 min by reading every 30 sec..

References

1. Chardin et al. 1996. Nature, 384, 481-484.
2. Stalder et al. 2011. JBC, 286 (5), 3873–3883.
3. Santy and Casanova. 2001. J. Cell Biol. 154, 599–610.
4. Viaud et al. 2007. PNAS, 104, 10370–10375.
5. Beemiller et al. 2006. PLoS Biol. 4, e162.
6. Cohen et al. 2007. MBC, 18, 2244–2253.
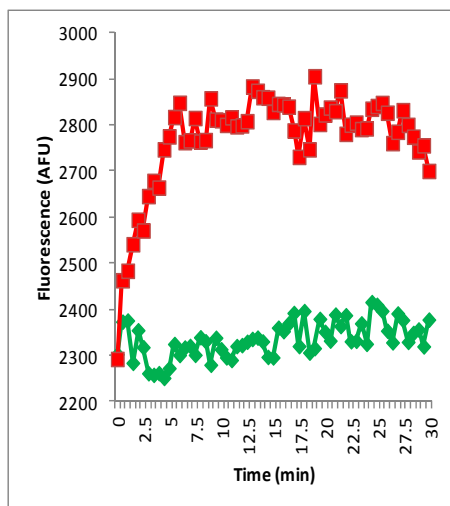7. Fuss et al. 2006. Nature, 444, 945–948.

Product Uses

- Study of ARNO interacting proteins, such as ARf proteins.
- Identification of ARNO interacting proteins.
- Drug discovery tool for Arf/ARNO pathway inhibitors.
- Positive control for Western blots.

Product Citations/Related Products

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