V. 1.1

**His-Arf1 Protein: amino acids 18-181**
**(Human recombinant)**
**Cat. # FR01 Amount = 100 µg**

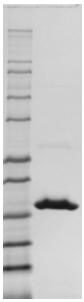
**Upon arrival store at 4°C (desiccated)**

**Material**
Human Arf1 protein (Acc.# NP_001019399), lacking the N-terminal 17 amino acids, has been produced in a bacterial expression system. The recombinant protein contains six histidine residues at its amino terminus (His-tag). The molecular weight of His-Arf1 is approximately 21 kDa and is supplied as a white lyophilized powder.

**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 (2 µl water per 10 µg protein). When reconstituted, the protein will be in the following buffer: 20 mM Tris pH 7.6, 0.5 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.

**Purity**
Protein purity is determined by scanning densitometry of Coomassie Blue stained protein on a 4-20% polyacrylamide gradient gel. His-Arf1 protein was determined to be 80% pure. (see Figure 1).

**Figure 1. His-Arf1 Protein Purity Determination.**




**Legend:** A 20 µg sample of recombinant His-Arf1 protein (molecular weight approx. 21 kDa) was separated using 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.

**Biological Activity Assay**
The biological activity of His-Arf1 can be determined from its ability to catalyze the exchange of GDP for GTP. The human ARNO protein is an exchange factor for Arf1, and is used with the Rho-GEF exchange assay biochem kit (Cat. # BK100) to monitor the exchange ability of His-Arf1. Note: Bodipy-FL-GDP or GTP can also be used to follow exchange with a large signal to noise ratio. Stringent quality control ensures that the exchange rate (Vmax) of Arf1 is enhanced two fold under the assay conditions described below.

**Reagents**
1. Recombinant His-Arf1 protein (Cat. # FR01)
2. Recombinant His-ARNO protein (Cat. # GE07)
3. 2x Exchange Buffer (40 mM Tris pH 7.5, 300 mM NaCl, 20 mM $MgCl_2$ , 2 mM DTT, 100 µg/ml BSA, and 1.5 µM mant-GTP).

The 2x Exchange Buffer is available in the RhoGEF exchange assay biochem kit (Cat # BK100).

**Equipment**
1. Fluorescence spectrometer. Program the fluorimeter at an excitation filter wavelength of 360 nm and emission filter wavelength of 440 nm. The bandwidth of the filter should be no more than 20 nm or significant background noise and reduced sensitivity of the assay may be experienced. The fluorimeter should be at 20°C and set on kinetic mode, it is recommended to take a reading once every 30 seconds for at least 60 cycles. We recommend a TECAN SpectroFluro plus (GmbH, Austria) or Perkin-Elmer LS spectrometer.
2. Corning 96-well half area plates (Cat. # 3686) or other plate with low protein binding surface.

**Method**
1. Resuspend the His-ARNO protein (Cat. # GE07) to 2 mg/ml (50 µM) with the addition of 25 µl nanopure water. Dilute an aliquot to 10 µM with nanopure water. Keep on ice.
2. Resuspend the His-Arf1 protein as described in the reconstitution section for a 200 µM solution. Dilute an aliquot to 50 µM with Milli-Q water. Keep on ice.
3. Add the following components together into four wells of a 96 well half area plate. Two wells will be the control reactions, and the others the test samples. Mix the components by gentle pipeting.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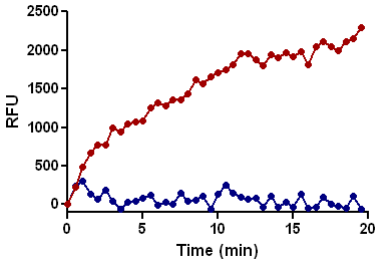
| Volume per well | Reagent |
|---|---|
| 75 µl | 2x Exchange buffer |
| 15 µl | 50 µM His-Arf1 |
| 57 µl | nanopure water |

4. Insert the plate into the fluorimeter and begin reading.
5. After 5-10 cycles (150-300 seconds, you can set this time as time zero), add 3 µl of the ARNO protein (10 µM) to the test wells and 3 µl of nanopure water to the control wells. Quickly mix the solutions by swirling with the tip or use the automix function where available. It is important to keep this mixing step as short as possible to obtain a smooth curve. Resume reading for at least 20 minutes.
6. The exchange rate can be calculated by reducing the data to Vmax with software that accompanies the plate reader. The exchange curve can be generated by exporting the raw data to Microsoft Excel.
7. A typical exchange curve is shown in Figure 2.

**Figure 2. Arf1 / ARNO exchange assay.**




Legend: Arf1 protein (5 µM) was mixed with exchange buffer and aliquoted to two wells of a 96-well half area plate. After 5 cycles of reading in a fluorimeter, ARNO protein (0.2 µM) or Milli-Q water were added to the wells and the reactions were monitored for 30 min as described in the method. Red circles represent the data with His-ARNO and the blue circles represent the control reactions lacking His-ARNO. Note: Bodipy-FL-GDP or GTP can also be used to follow exchange with a large signal to noise ratio.

**Product Uses**

- Study of Arf1 interacting proteins, Effectors, GAPs and GEFs.
- Identification of Arf1 interacting proteins.
- Positive control for Western blots.

**Product Citations/Related Products**

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