Data Sheet

GFP, FLAG-tagged SNRNP70 mRNA

Synthetic RNA

03-903

Pack Size 50 µg

Store at -80 °C

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Both mRNAs and non-coding RNAs interact with RNA-binding proteins and regulate critical cellular processes and their dysregulation contributes to multiple diseases. Thus, analyzing RNA-protein interactions are critical for understanding the mechanism of diseases and designing clinical strategies.

RNA-binding protein immunoprecipitation (RIP) has been used to interrogate RNA binding proteins (RBP) associated with specific RNA molecules. However, the experiments are not feasible because of the unavailability of antibodies for the particular RBP or low expression of the RBP. To overcome these issues epitope tags have been used for RIP experiments.

The GFP, FLAG-tagged SNRNP70 mRNA was developed to enable to perform control RIP experiments with RIPAb+ FLAG (03-901) or RIPAb+ GFP (03-902) RIP validated antibody and primmer kits, and Magna RIP® Kit (17-700).

The high amount of GFP-FLAG-tagged SNRNP70 protein is expressed immediately after transfection to cells and the well-known specific interaction of SNRNP70 and U1snRNA can be used for control RIP experiments with anti-GFP or anti-FLAG antibodies.

GFP-tag and FLAG-tag sequences are fused to the 3’ of the SNRNP70 mRNA. The GFP tag uses TagGFP2 an improved variant of the Aequorea macrodactyla GFP-like protein. TagGFP2 exhibits bright green fluorescence comparable to that of EGFP, with excitation/emission maxima at 483 and 506 nm, respectively. The anti-Green Fluorescent Protein Antibody in the RIPAb+ GFP (03-902) interact with both GFP and tagGFP2.

Source

In vitro synthesized RNA

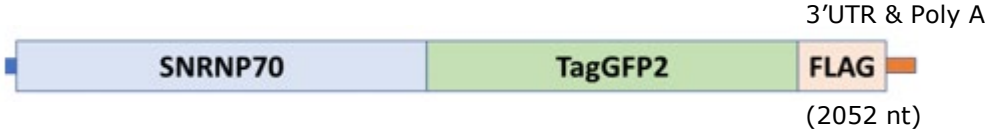

Figure 1. Structure of GFP, FLAG-tagged SNRNP70 mRNA.

Storage and Handling

Store at: -80 °C

Presentation

GFP, FLAG-tagged SNRNP70 mRNA: (03-903) One (1) vial containing 50 µL of RNA (1 µg/µL)

Quality Control Testing

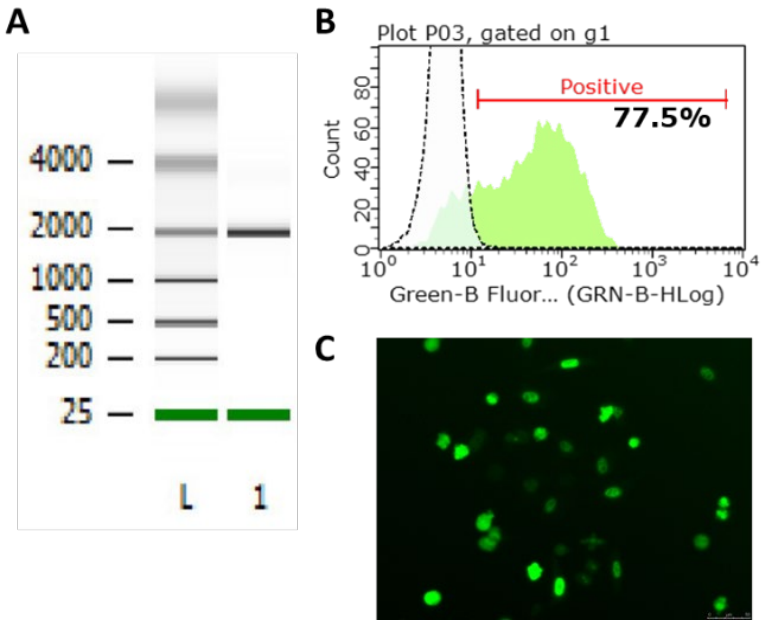

Figure 2. Quality of the RNA with Bioanalyzer and the Expression of TagGFP2 in Hela Cells

(A) GFP, FLAG-tagged SNRNP70 mRNA was analyzed with Bioanalyzer. L: RNA ladder, 1: mRNA, (B, C) GFP, FLAG-tagged SNRNP70 mRNA was transfected into HeLa cells. One day after transfection, GFP expression was analyzed by flow cytometry and fluorescence microscopy.

Materials Required (Not Supplied)

- RiboJuice™ mRNA Transfection Kit (TR-1013)
- RIPAb+ FLAG (03-901) or RIPAb+ GFP (03-902) RIP validated antibody and primer kits
- Magna RIP® Kit (17-700)

Representative Data

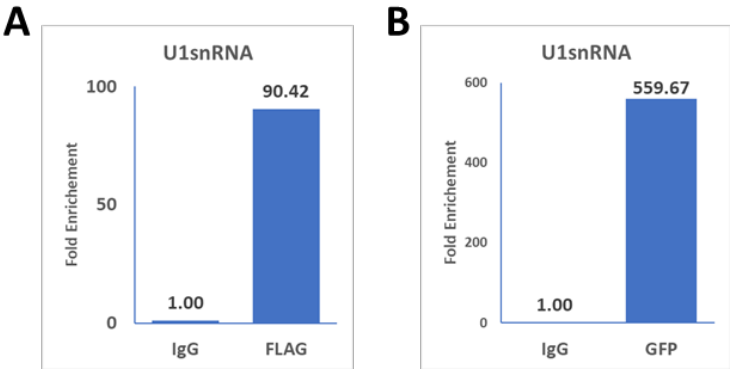

Figure 3. RNA Binding Protein Immunoprecipitation

RIP Lysate was prepared from HeLa cells (1 X 10^7 cell equivalents per IP) expressing SNRNP70-GFP-FLAG (03-903) by RNA transfection. The lysate was subjected to immunoprecipitation using (A) 5 µg of either a normal mouse IgG (CS200621) or Anti-FLAG M2 antibody (CS226432), and (B) 5 µg of either a Rabbit IgG purified (PP64B) or Anti-Green Fluorescent Protein Antibody (AB3080) with the Magna RIP® RNA-Binding Protein Immunoprecipitation Kit (17-700). Successful immunoprecipitation of SNRNP70-GFP-FLAG associated RNA was verified by qPCR using RIP Primers, U1 snRNA, (CS203215)

Note: Please refer to the Magna RIP® (17-700) or EZ-Magna RIP® (17-701) protocol for experimental details.

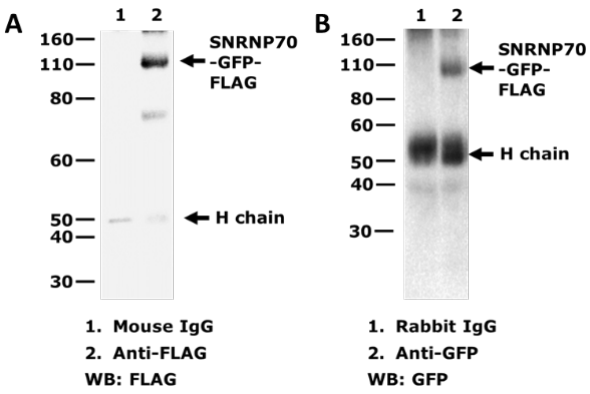

Figure 4. Immunoprecipitation from RIP lysate

RIP Lysate was prepared from HeLa cells (1 X 10^6 cell equivalents per IP) expressing SNRNP70-GFP-FLAG (03-903) by RNA transfection. The lysate was subjected to immunoprecipitation using (A) 0.5 µg of either normal rabbit IgG, (CS200621) or Anti-FLAG M2 Antibody (CS226432), (B) 0.5 µg of either Rabbit IgG purified (PP64B) or Anti-Green Fluorescent Protein Antibody (AB3080). Precipitated proteins were resolved by electrophoresis, transferred to nitrocellulose, and probed with (A) anti-FLAG M2 (1.0 µg/mL) or (B) Anti-Green Fluorescent Protein Antibody (1.0 µg/mL). Proteins were visualized using (A) a goat anti-mouse secondary antibody conjugated to HRP (AP124P) or (B) a goat anti-Rabbit secondary antibody conjugated to HRP (AP187P), and a chemiluminescence detection system.

Transfection Protocol

Transfection of GFP, FLAG-tagged SNRNP70 mRNA has been validated using RiboJuice™ mRNA Transfection Kit (TR-1013). The amounts of RNAs and transfection reagents required may vary depending on the target cells. Here is an example to transfect HeLa cells on a 10 cm plate (~1.0 x 10^7 cells). 5 to 10 µg of the mRNA per plate would be used.

1. Plate cells to reach 50-90% confluency at the time of transfection.
2. Wash cells once with EMEM (no serum, no antibiotics) and add 5 mL/plate of EMEM (no serum, no antibiotics). No serum condition increases the transfection efficiency. However, it is possible to use 1-10% serum depending upon cell types. Incubate cells in a 37 °C, 5% CO_2 incubator (10-20 minutes).
3. Set up transfection reactions in sterile Eppendorf tubes following the table. Follow the order of additions. Mix gently by pipetting during each addition of RNA and reagent. Do not vortex.

RiboJuice™ mRNA Transfection Kit

Component	5 µg	10 µg
Opti-MEM® I Reduced-Serum Medium (Life Technologies, 31985062)	500 µL	1000 µL
B18R-E3L RNA (1 µg/µL)	5 µL	10 µL
RiboJuice™ mRNA Boost Reagent	10 µL	20 µL
RiboJuice™ mRNA Transfection Reagent	10 µL	20 µL
Total Volume	**525 µL**	**1050 µL**

4. Incubate the tubes at room temperature for 5 minutes and add the RNA-transfection reagent complex dropwise into plates containing cells.
5. Incubate the plate in a 37 °C, 5% CO_2 incubator for 2-4 hours. Maximum transfection efficiency is obtained with 4 hours of incubation using RiboJuice™ mRNA Transfection Kit with Hela cells.
6. Aspirate the transfection medium and add 10 mL per plate of EMEM containing 10% FBS. Incubate in a 37 °C, 5% CO_2 incubator overnight.
7. Next day, Check the expression of GFP-FLAG tagged protein with a fluorescent microscope. Harvest the cells and perform RIP assay with Magna RIP® Kits and RIPAb+ antibodies.

Related Products

Description	Catalogue Number
RIPAb+ EZH2, clone AC22	03-900
RIPAb+ FLAG	03-901
RIPAb+ GFP	03-902
Magna RIP® RNA-Binding Protein Immunoprecipitation Kit	17-700
Magna RIP® Quad RNA-Binding Protein Immunoprecipitation Kit	17-704
EZ-Magna RIP® RNA-Binding Protein Immunoprecipitation Kit	17-701

Description	Catalogue Number
Anti-Ago1, clone 6D8.2	04-083
Anti-Ago 2	07-590
Anti-Ago family	04-085
Anti-pan Ago, clone 2A8	MABE56
Anti-Ago1, clone 4G7-E12	MABE143
Anti-Ago3, clone 4B1-F6	MABE144
RIPAb+™ pan Ago	03-248

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