GENE CREATE

RNA Binding Protein Immunoprecipitation (RIP)Kit

Catalog# JKR23003-12T

Instruction Manual (For Two Groups)

Sufficient reagents for 12 RIP assays per kit.
Store at -20 & 4°C

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1. Experimental Principle

RIP technology (RNA Binding Protein Immunoprecipitation) is a technique used to study the binding of RNA and proteins within cells, serving as a powerful tool for understanding the dynamic processes of post-transcriptional regulatory networks. This method primarily employs specific antibodies against target proteins to precipitate the corresponding RNA-protein complexes, followed by isolation and purification to validate the bound RNA via q- PCR or high-throughput sequencing analysis.

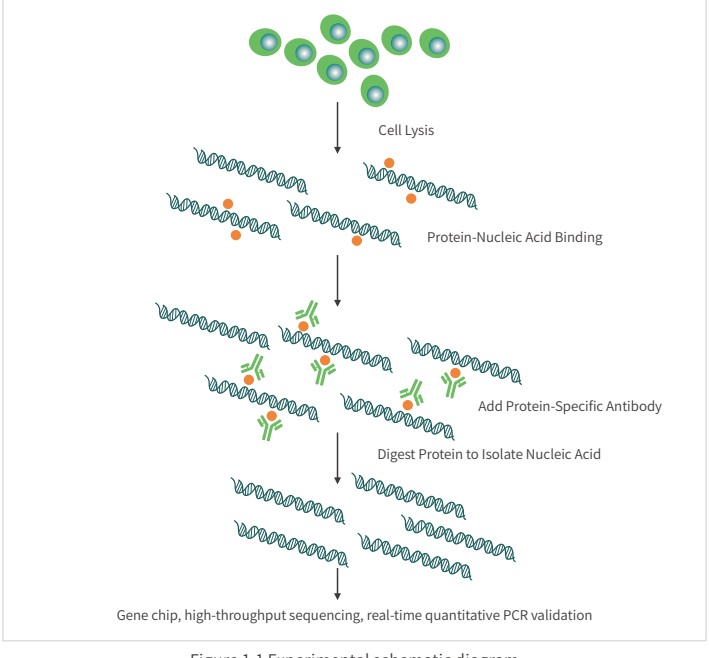
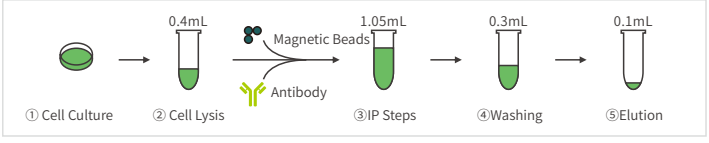


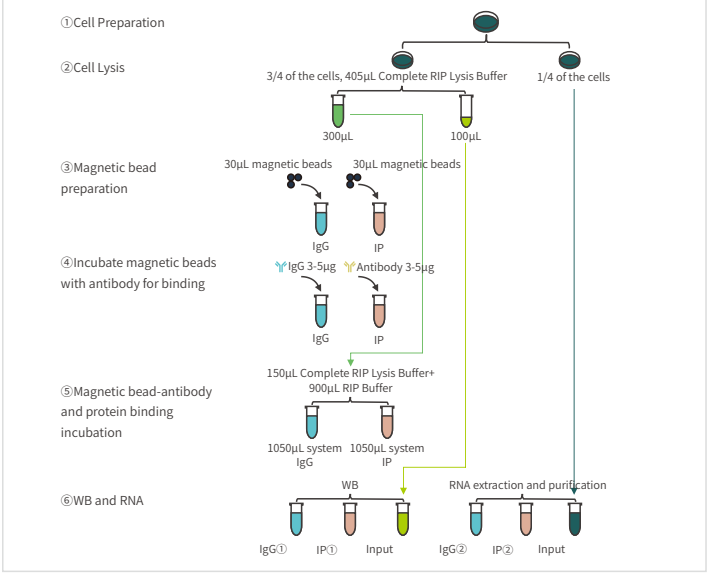
Figure 1.1 Experimental schematic diagram

2. Technical Roadmap

2.1 Experimental Flowchart



2.2 Control Setup Flowchart



3. Kit Components

Component	Volume (12T)	Storage Temperature
RIP Lysis Buffer	7.2mL	4°C
Protease Inhibitor (100X)	30μL	-20°C
RNase Inhibitor	70μL	-20°C
Protein A/G Magnetic Beads	400μL	4°C
Normal Rabbit IgG (1mg/mL)	60μL	-20°C
Normal Mouse IgG (1mg/mL)	60μL	-20°C
RIP Wash Buffer	100mL	4°C
0.5 M EDTA	800μL	4°C
Salt Solution	1.2mL	4°C
DEPC H_2O	800μL	4°C
Elution Buffer	1.6mL	Protect from light at 4°C

Special note 1: Trizol, 75% ethanol, chloroform, isopropanol, reverse transcription kit, and fluorescent dye must be self-prepared;
Special Note 2: 12T corresponds to 12 individual immunoprecipitation experiments (1 IP group or 1 IgG group). The subsequent procedure includes one IgG group and one IP group, consuming 2T of reagent.

4. Operating Steps

4.1 Sample Lysis

4.1.1 Animal Cells

① Prepare 405 μL Complete RIP Lysis Buffer (400 μL RIP Lysis Buffer + 4 μL Protease Inhibitor (100X) + 1μL RNase Inhibitor);

② Collect a $2x10^7$ cell sample, add 2 mL PBS to wash the cells, centrifuge and discard the supernatant to collect the cell pellet. Reserve 1/4 of the cell sample for subsequent RNA extraction and purification of the input group (store in nuclease-free EP tubes at -80°C);

③ Add 400 μL of Complete RIP Lysis Buffer to the remaining cell pellet and resuspend by pipetting up and down 10 times to ensure complete lysis. Incubate the lysate on ice for 30 min, vortexing for 10 sec at 5-min intervals. Subsequently, perform

sonication on ice for 5 min using an ultrasonic cell disruptor at 20% power (3 sec on, 3 sec off). Centrifuge at 12,000 rpm and 4°C for 10 min. Collect the supernatant, label it as “Lysis”, and aliquot into three parts: 150 μL (for IP), 150 μL (for IgG), and 100 μL (for Input). Store the Input aliquot at -80°C for future use.

4.1.2 Animal Tissue

① Prepare 405 μL Complete RIP Lysis Buffer (400 μL RIP Lysis Buffer + 4 μL Protease Inhibitor (100X) + 1μL RNase Inhibitor);

② Grinding: Take fresh or cryopreserved tissue (approximately 0.3 g), place it in a sterilized and pre- cooled mortar, add liquid nitrogen and quickly grind into a powder. Collect about 1/4 of the sample and reserve it for subsequent RNA extraction and purification of the input group (store in nuclease- free EP tubes at -80°C);

③ Add 250 μL of Complete RIP Lysis Buffer to the remaining sample in the mortar, continue grinding on ice for 5-10 min until the sample becomes a fine homogenate, transfer to a new EP tube.Then add the remaining 150 μL of Complete RIP Lysis Buffer to the mortar to collect residual sample, and also transfer it to the same EP tube;

④ The EP tube containing the sample homogenate is fully lysed on ice for 30 min , vortexed every 5 min for 10 sec each time. After lysis, perform ice-bath sonication using a cell ultrasonic disruptor for 8-10 min at 20% power, with 3 sec of sonication and 3 sec of intervals.

⑤ Centrifuge at 4°C, 12,000 rpm for 10 min , collect the supernatant. Then add RIP Lysis Buffer to the supernatant to bring the volume to 400 μL, mix well, and divide into three portions: 150 μL (IP), 150 μL (IgG), and 100 μL (Input). Store the Input sample at -80°C for later use.

4.2 Prepare magnetic beads

① Prepare two 1.5 mL EP tubes, label them as the IgG tube and IP tube respectively. Invert the original Protein A/G Magnetic Beads tube 10 times, and after the beads and liquid are fully mixed, transfer 30 μL each to the IgG tube and IP tube;

② Add 300 μL of RIP Wash Buffer to each tube, mix by pipetting up and down 5 times, place on a magnetic stand for 1min, and discard the supernatant. Repeat this step 3 times;

③ Resuspend the beads with 300 μL of RIP Wash Buffer.

4.3 Magnetic Bead-Antibody Conjugation

① Add 3-5 μg of target antibody to the IP tube, and add 3-5 μg of IgG from the same host as the target antibody to the IgG tube; incubate on a silent mixer at room

temperature for 2 h;

② Place both tubes on a magnetic stand for 1 min, then discard the supernatant;

③ Add 300 μL of RIP Wash Buffer, mix by pipetting up and down 5 times, let stand on the magnetic stand for 1 min, discard the supernatant, and repeat this step 3 times.

4.4 RNA-Binding Protein Immunoprecipitation

① Prepare 1.8 mL of RIP Buffer (1720 μL RIP Wash Buffer + 70 μL 0.5 M EDTA+10 μL RNase Inhibitor);

② Add 900 μL RIP Buffer and 150 μL Lysis from step 4.1 to the IgG tube and IP tube obtained in step 4.3, respectively, place on a silent mixer, and incubate overnight at 4°C;

③ Place both tubes on a magnetic stand for 1 min, then discard the supernatant;

④ Add 300 μL RIP Wash Buffer to each tube, pipette up and down to mix 5 times, let stand on the magnetic stand for 1 min, discard the supernatant, and repeat this step 5 times;

⑤ Add 300 μL RIP Wash Buffer to each tube, mix by pipetting up and down 5 times, transfer 100 μL of the mixture to a new tube labeled as tube ①, and designate the remaining liquid as tube ②. Place all four tubes on a magnetic stand for 1 min, discard the supernatant, Add 100 μL Elution Buffer to tube ①, boil for 10 min, then place on the magnetic stand for 1 min, Transfer the supernatant to a new tube, add 10 μL 6X Loading buffer, mix, and prepare for WB detection, Tube ② is used for RNA purification. Group settings are shown in the table below:

Group	RIP Wash Buffer	Name	Volume	Purpose
IgG	300μL	IgG-①	100μL	WB
		IgG-②	200μL	RNA purification
IP	300μL	IP-①	100μL	WB
		IP-②	200μL	RNA purification

4.5 RNA Purification

① Add 500 μL of Trizol to the input sample reserved in step 4.1, the IgG-② tube, and the IP-② tube, vortex to mix, let stand at room temperature for 5 min, then add 100 μL of chloroform, vortex to mix, centrifuge at 14,000 rpm for 10 min at 4°C, transfer the upper aqueous phase (approximately 300 μL) to a new tube, and label it;

② Add 50 μL of Salt Solution and 550 μL of isopropanol, mix well, let stand at -80°C for 2–4 h (or overnight), thaw at 4°C before use;

③ Centrifuge at 14,000 rpm for 10 min at 4°C, discard the supernatant;

④ Add 500 μL of 75% ethanol, centrifuge at 14,000 rpm for 10 min at 4°C, carefully discard the supernatant, repeat this step three times;

⑤ Open the lid and air-dry at room temperature, add 10-20 μL DEPC H2O, pipette to mix thoroughly until RNA is completely dissolved, and store at -80°C for future use.

4.6 RIP-qPCR (Optional)

① Reverse transcription: Perform according to the reverse transcription kit instructions.

② Reaction procedure: Add 1 μL of cDNA from Input, IP, and IgG after reverse transcription into PCR reaction wells, with three replicates per sample. Add remaining components as per the SYBR Green qPCR Mix instructions. After addition, label and briefly centrifuge for 10sec, then place in the real-time PCR instrument for detection. Detailed loading method is as follows (for positive samples, only GAPDH primers are required to confirm enrichment):

		1	2	3	4	5	6	7	8	9
Primer1	B	Input	Input	Input	IgG	IgG	IgG	IP	IP	IP
Primer2	C	Input	Input	Input	IgG	IgG	IgG	IP	IP	IP
...	...	Input	Input	Input	IgG	IgG	IgG	IP	IP	IP

③ Result calculation: Fold Enrichment = 2^(-ΔΔCt) (using IgG as reference)

4.7 RIP-seq (optional)

Take an appropriate amount of Input and IP samples and proceed according to the RNA library preparation kit instructions.

5. Frequently Asked Questions

Q1: How to determine if the RIP experiment is successful?

The RIP experiment is considered successful if the target protein signal is detected in both the IP group and the input group by WB assay.

Q2: No difference in Ct values between IP and IgG samples

Caused by various factors: 1.The antibody did not enrich RNA—try replacing the antibody; 2.High IgG background—increase washing times or reduce RNA input in the immunoprecipitation step.

Q3: Abnormal melting curve

If non-specific amplification or primer dimer formation occurs, redesign the primers.

Q4: Pull-down sample RNA concentration is too low

1.Insufficient sample input, consider increasing the initial sample amount; 2.Incomplete lysis, tissue samples not fully ground, or use a strong lysis buffer for lysis.

WUHAN GENECREATE BIOLOGICAL ENGINEERING CO.,LTD.

Address： Wuhan Shengzhiyuan Biotechnology Innovation Industrial Park,
North Shendun 5 th Road, Jiangxia District, Wuhan City, Hubei Province.
Phone: 027-87960366
Email: marketing@genecreate.com
Website: www.genecreate.cn