RIP Assay Kit

Cat. No. KITZ-068 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	The mRNA encoding proteins that play roles in specific cellular processes or pathways can be found in a unique mRNP complex, consisting of mRNA and RNP. This not only provides information about the known components of specific processes or pathways but also helps identify new components that represent potential therapeutic targets and biomarkers. To better study the components of mRNP complexes, RIP-Chip technology was developed. These techniques allow the separation and identification of the mRNA, microRNAs, and the protein components of RNP complexes from cell extracts using antibodies against RBPs and microarrays. RIP Assay Kit is an optimized version of the RIP-Chip process. The kit is free of phenol and chloroform, allowing for the safe isolation of "high-quality RNA" from RNP complexes without degradation. The purified RNA is suitable for various analytical methods such as RT-PCR and sequencing. Compared to traditional RNA isolation and analysis methods, the fractionation process of the RIP Assay Kit effectively concentrates RNA species bound to a specific binding protein, enabling the detection of subtle changes in low-abundance RNA levels with a greatly improved signal-to-noise ratio. Another advantage of the RIP Assay Kit is the minimization of RNA recombination, allowing for significant recovery of RNA contained in the RNP complexes of interest.
Size	10 Assays
Storage	Kit is stable for 2 years from the date of manufacture when stored at 4°C. Do not freeze.

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Shipping	Ice pack
Kit Components	1. Lysis Buffer: 26 mL × 1 bottle. 2. Wash Buffer: 35 mL × 2 bottles. 3. Normal Rabbit IgG: 0.2 mL × 1 vial. Negative control: 200 μg of normal rabbit IgG in 200 μL of phosphate buffered saline (PBS) containing 50% Glycerol (pH 7.2). 4. High-Salt Solution: 6 mL × 1 vial. In some cases, addition of this solution to both Lysis Buffer and Wash Buffer is required. 5. Solution I: 0.26 mL × 1 vial: enzyme solution 6. Solution II: 10 mL × 1 vial: diluent for Solution I 7. Solution III: 7 mL × 1 vial: protein dissolvent. Solution III can dissolve proteins and dissociate immunocomplex. 8. Solution IV: 55 μL × 1 vial: co-precipitator. Solution IV can increase RNA precipitation efficiently. Note: 1. Solution II may become turbid when stored for long-term at 2-8°C. Turbidity does not affect performance. If Solution II is turbid, equilibrate to room temperature (15-25°C) and mix well before use. 2. Precipitates may appear when Solution III is stored for long-term at 2-8°C. If Solution III contains precipitates, dissolve them by equilibrating the solution to room temperature (15-25°C) and mix well before use. 3. This reagent contains guanidine hydrochloride; this is a potentially hazardous substance and should be used with appropriate caution.
Materials Required but Not Supplied	1. RIP-Certified Antibody 2. Microcentrifuge capable of 15,000 × g 3. Microcentrifuge tube (1.5mL or 2mL) (Nuclease-free) (Recommendation: use low-adhesion tube for RIP-Assay) 4. Centrifuge capable of 2,000 × g 5. Centrifuge tube (15 mL or 50 mL)

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6. Pipettes (5 mL, 10 mL, 25 mL) (Nuclease-free)
7. Pipette tips (10 µL, 20-100 µL, 200 µL, and 1,000 µL) (Nuclease-free)
(Recommendation: use low-adhesion pipette tip for RIP-Assay)
8. Ultra-low temperature freezer (-80°C)
9. Freezer (below -20°C)
10. End-over-end rotator
11. Vortex mixer
12. Gloves
13. Protease inhibitor (molecular biology grade)
Commercial reagent: Aprotinin, Leupeptin, Phenylmethylsulfonyl fluoride (PMSF)
14. RNase inhibitor
15. Dithiothreitol (DTT)
16. Protein A or Protein G Agarose beads
17. 100% Ethanol (molecular biology grade)
18. 100% 2-Propanol (molecular biology grade)
19. Nuclease-free PBS
20. Nuclease-free water
21. Isotype control IgG (if necessary)
Note:
* Recommended concentration of each reagent is shown in Appendix.
** Commercially available reagents confirmed to work with RIP-Assay Kit are shown in
Appendix.
*** In the case of using monoclonal antibodies for RNP immunoprecipitation, the isotype
control IgG should be prepared as a negative control.

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