Protein-RNA ELISA Assay

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[Abstract] Protein-RNA ELISA assay is an effective and quantitative method to study protein-RNA interactions *in vitro*. In this protocol we used recombinant 6x HIS tagged protein, but it works as well for non tagged proteins.

Materials and Reagents

1. 96-well Microplate Nunc maxisorp White (Thermo Fisher Scientific, Nunc, catalog number: 436110)
2. Recombinant 6x HIS tagged protein
3. Streptavidin (New England Biolabs, catalog number: N7021S)
4. $NaHCO_3$
5. Phosphate buffered saline (PBS) (Life Technologies, Gibco®, catalog number: 10010031)
6. Tween 20
7. BSA
8. Biotinylated RNA
9. 1 M Tris (pH 7.5)
10. NaCl
11. Yeast tRNA (Life Technologies, Applied Biosystems®, catalog number: AM7119)
12. 6x His mAb-HRP Conjugate (Clontech, catalog number: 631210)
13. ECL Peroxidase Substrate Solution A and B (Pierce Antibodies, catalog number: 32106)
14. Water used for all solution is RNAase free
15. q-PCR tape (R&D systems, catalog number: DY992)
16. RiboLoc RNAase Inhibitor (Thermo Fisher Scientific, catalog number: EO0381)
17. RNA 3' End Biotinylation Kit (Pierce Antibodies, catalog number: 20160)
18. Binding buffer (see Recipes)
19. PBS-T (see Recipes)

Equipment

1. Luminescence Plate reader (BMG LABTECH, FLUOstar OPTIMA)
2. Thermocycler
3. Centrifuges

Procedure

1. Coated 96-well plates with 50 µl/well of streptavidin (100 µg/ml in 0.1 M $NaHCO_3$).
2. Incubate overnight at 4 °C. Seal the wells with q-PCR tape.
3. Wash plates six times with 200 µl PBS-T (there is no need to incubate for washing).
4. Block with 50 µl/well of PBS containing 3% BSA and 0.1 µg/ml streptavidin.
5. Incubate overnight at 4 °C or 7 h at RT. Seal the wells with q-PCR tape (for my experience, in this step overnight or at least 7 h RT incubation is needed as less time causes high noise).
6. Mix in PCR tubes biotinylated RNA (5 pmol) and different amounts of protein (0-500 ng) in binding buffer. The final volume in each tube is 50 µl. The RNA was biotinylated using the RNA 3' End Biotinylation Kit.
7. Incubate the PCR tubes with the biotinylated RNA plus the protein, in a thermocycler for 30 min at 37 °C.
8. Wash plates four times with 200 µl PBS-T.
9. Transfer the mix (biotinylated RNA-protein) in to the plates (50 µl/well) and incubate 1 h at RT. Seal the wells with q-PCR tape (No need to rock to rock/shake the plate).
10. Wash plates six times with 200 µl/well PBS-T.
11. Add the 6x His mAB/HRP conjugate in PBS 1:1,000 (50 µl/well) incubated for 1 h at RT. Seal the wells with q-PCR tape
12. Wash plates six times with 200 µl/well PBS-T.
13. Add 50 µl/well of a mix 1:1 ECL (25 µl of ECL1 and 25 µl of ECL2).
14. Spin the plate to avoid bubbles using an adaptor for a swing bucket centrifuge.
15. Read luminescence in the Labtech FLUOstar OPTIMA (MARS Software from labtech on Nunc maxisorp 96. 0.5 second interval).

Recipes

1. Binding buffer
   50 mM Tris (pH 7.5)
   150 mM NaCl

0.02 mg/ml yeast tRNA
0.2 mg/ml BSA
1.5 µl RiboLoc RNAase Inhibitor
RNAase free water
In a total volume of 50 µl per well

2. PBS-T
   1x PBS
   0.1% Tween 20

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