Please cite this article as: Yan and Xiaochen, (2013). *In vitro* Lipid Binding Assay, *Bio-protocol* 3 (9): e694. DOI: 10.21769/BioProtoc.694.

In vitro Lipid Binding Assay

Yan Zhang and Xiaochen Wang*

National Institute of Biological Sciences, Zhongguancun Life Science Park, Beijing, China
*For correspondence: wangxiaochen@nibs.ac.cn

[Abstract] This is a protocol to examine *in vitro* protein-lipid binding using membrane strips coated with various lipids. It has been successfully used to study *in vitro* interaction between lipids and *C. elegans* proteins.

Materials and Reagents

1. Membrane Lipid strip (Echelon Bioscience, catalog number: P-6001)
2. Proteins of interest (EGFP-NRF-5-Flag, EGFP-Flag as control)
3. Anti-Flag M2 antibody (Sigma-Aldrich, catalog number: A2220)
4. Primary antibodies (Anti-Flag M2 from Mouse, sigma, catalog number:F1804)
5. HRP-conjugated secondary antibodies (*e.g.* Goat anti-Mouse LgG (H+L) HRP, The Jackson Laboratory, catalog number: 115-035-003)
6. SuperSignal West Pico reagent (Thermo Fisher Scientific, catalog number: 34080)
7. 293T cells
8. Tris-Cl
9. NaCl
10. Tween-20
11. Ca^{2+} chloride
12. Zn^{2+} sulfate
13. Non-fat milk
14. Blocking buffer (see Recipes)
15. Incubation buffer (see Recipes)
16. Wash buffer (see Recipes)

Equipment

1. Vortexer
2. Rotator

Please cite this article as: Yan and Xiaochen, (2013). *In vitro* Lipid Binding Assay, *Bio-protocol* 3 (9): e694. DOI: 10.21769/BioProtoc.694.

Procedure

1. Membrane strips coated with various lipids are incubated in 10 ml of blocking buffer for 1 h at room temperature (RT) in dark (the commercially available membrane strip has been coated with lipids).
2. 20-60 μg of proteins are added to 6 ml incubation buffer with membrane strips and incubated overnight at 4 °C. In our study, we used Flag-tagged protein purified from 293T cells (Zhang *et al.*, 2012). However, proteins purified from other sources can all be used. Negative controls should be included according to specific proteins used in this assay.
3. Membrane strips are washed extensively with wash buffer for 3 times at RT, 10 min each wash on rotator.
4. Membrane strips with bounded proteins are incubated with primary antibodies (Anti-Flag M2 from mouse, 1:1,000) in incubation buffer for 3 h at RT.
5. Wash 3 times at RT with wash buffer, 10 min each wash on rotator.
6. Incubate membrane strips with HRP-conjugated secondary antibodies (Goat anti-Mouse LgG (H+L) HRP, 1:10,000) in incubation buffer for 1 h at RT.
7. Membranes are washed as above and protein-antibody interaction is detected using SuperSignal West Pico reagent.

Recipes

1. Blocking buffer
   25 mM Tris-Cl
   150 mM NaCl
   0.1% Tween-20
   1% non-fat milk
2. Incubation buffer
   25 mM Tris-Cl
   150 mM NaCl
   0.1% Tween-20
   1% non-fat milk
   2 mM Ca^{2+} chloride
   1 mM Zn^{2+} sulfate
3. Wash buffer
   25 mM Tris-Cl
   150 mM NaCl
   0.1% Tween-20
   2 mM Ca^{2+} chloride
   1 mM Zn^{2+} sulfate

bio-protocol http://www.bio-protocol.org/e694 Vol 3, Iss 9,May 05, 2013

Acknowledgments

This protocol is adapted from Zhang *et al*. (2012) and Wang *et al*. (2010).

References

1. Wang, X., Li, W., Zhao, D., Liu, B., Shi, Y., Chen, B., Yang, H., Guo, P., Geng, X., Shang, Z., Peden, E., Kage-Nakadai, E., Mitani, S. and Xue, D. (2010). *Caenorhabditis elegans* transthyretin-like protein TTR-52 mediates recognition of apoptotic cells by the CED-1 phagocyte receptor. *Nat Cell Biol* 12(7): 655-664.
2. Zhang, Y., Wang, H., Kage-Nakadai, E., Mitani, S. and Wang, X. (2012). *C. elegans* secreted lipid-binding protein NRF-5 mediates PS appearance on phagocytes for cell corpse engulfment. *Curr Biol* 22(14): 1276-1284.

