PTEN-lipid Binding Assay

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[Abstract] The lipid and protein interactions are an integral and important part of many cellular signaling pathways. The understanding of the selective and specific interaction of the given lipid molecule with the target protein is required for studying cellular signaling. In this assay, different lipids are spotted onto a nitrocellulose membrane to which they attach. Then the membrane is incubated with a lipid binding protein possessing an epitope tag. The protein binds to the lipid which is detected by immunoblotting with an antibody recognizing the epitope tag (see Figure 1). PTEN is an important tumor suppressor which functions as both protein and lipid phosphatase. The primary physiological substrate of PTEN is signaling lipid PtdIns (3, 4, 5) P3, by dephosphrylating PtdIns (3, 4, 5) P3 to PtdIns (4, 5) P2 PTEN negatively regulates PI3K signaling and mediates its tumor-suppressor function by inactivating downstream oncogenic AKT-mediated signaling. The PTEN lipid binding assay is conducted to study the specific binding of PTEN to different lipid molecules.

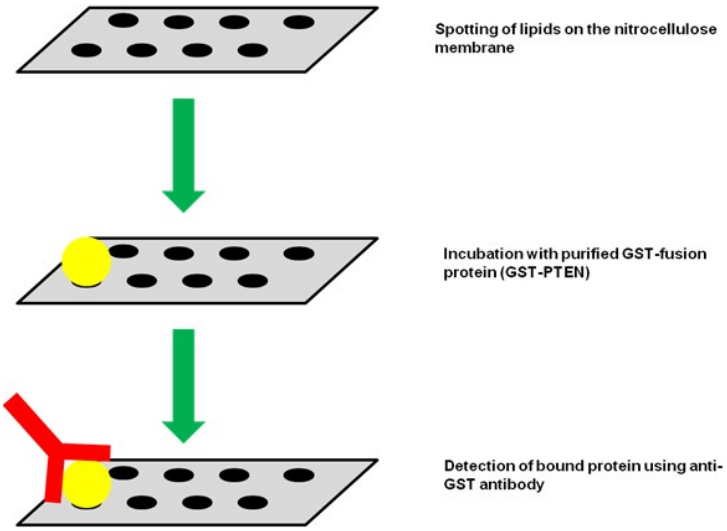

Figure 1. Key steps of the PTEN-lipid binding assay

Materials and Reagents

1. Lyophilized lipids:

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PE (Sigma-Aldrich, catalog number: P0890)
PC (Sigma-Aldrich, catalog number: P1652)

2. Hybond C-extra nitrocellulose membrane (Amersham Hybond-ECL, catalog number: RPN303D)
3. Bacterially Purified GST-fusion protein (GST-PTEN)
4. Anti-GST monoclonal antibody (Santa Cruz, catalog number: SC-138)
5. HRP-conjugated anti mouse secondary antibody (Jackson Immuno Research, catalog number: 315035048)
6. ECL (Thermo Scientific Prod, catalog number: 34080)
7. Methanol
8. Chloroform
9. 1x TBST buffer (see Recipes)
10. Blocking buffer (see Recipes)

Equipment

1. Shaker
2. Film developer

Procedure

1. Reconstitute the lyophilized lipids in a 2:1:0.8 solution of chloroform: methanol: water to make the required stock (all lipids were constituted to make stock of 1 mM).
2. Dilute the lipids to get the required working concentration (the working concentration used was 1 nM).
3. Spot 1 nM of the lipid dilution onto the Hybond C-extra nitrocellulose membrane (each spot is separated by ~1 cm).
4. Allow to dry at room temperature (RT) for 1 h.
5. Incubate the membrane with gentle rocking in blocking buffer for 1 h at RT.
6. Incubate the membrane overnight at 4 °C with gentle rocking in the fresh blocking buffer containing 20-100 nM of the GST-fusion protein (or other epitope tagged protein).
7. Wash the membrane 10 times over 50 min in TBST (use adequate volume of TBST which will cover the membrane ~10 ml).
8. Incubate the membrane for 1 h at RT with 1:1,000 dilution of the anti-GST monoclonal antibody in blocking buffer.
9. Wash the membrane 10 times over 50 min in TBST.

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10. Incubate the membrane for 1 h with a 1:10,000 dilution of the HRP-conjugated antimouse secondary antibody in blocking buffer at RT.
11. Wash the membrane 12 times over 60 min in TBST.
12. Detect the lipid binding protein bound to the membrane by ECL according to manufacturer's instructions (see Figure 2).

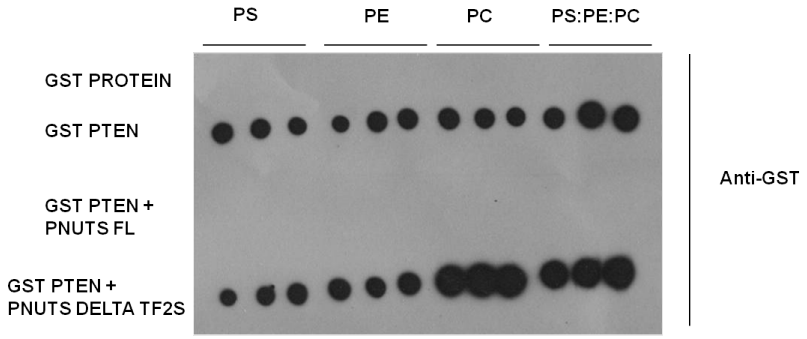

Figure 2. A blot representing the effect of PNUTS on the lipid binding property of PTEN. Nitrocellulose membranes spotted with phophatidylserine (PS) or phosphatidylethanolamine (PE) or phosphatidylcholine (PC) or PS: PE: PC mix (1:1:1) in triplicate was incubated with indicated recombinant proteins. Bound PTEN was detected with anti-GST antibody (adapted from Kavela *et al*., 2013)

Recipes

1. 1x TBST solution (1 L)
   50 mM Tris-HCl
   150 mM NaCl
   0.1% Tween 20
   Adjust pH 8 make up to 1 L
2. Blocking buffer
   3% BSA in 1x TBST

Acknowledgments

This protocol is adapted from Kavela *et al.* (2013).

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References

1. Kavela, S., Shinde, S. R., Ratheesh, R., Viswakalyan, K., Bashyam, M. D., Gowrishankar, S., Vamsy, M., Pattnaik, S., Rao, S., Sastry, R. A., Srinivasulu, M., Chen, J. and Maddika, S. (2013). PNUTS functions as a proto-oncogene by sequestering PTEN. *Cancer Res* 73(1): 205-214.

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