ELISA for tau binding to a protein of interest

Protocol developed by Zapporah Young/Meredith Kuo, modified by Taylor Arhar 022019

Introduction:

This assay is designed to determine apparent binding affinities between tau and an immobilized protein of interest. The protocol has been modified/optimized for binding to EB3.

Materials:

* Fisherbrand, Flat bottom 96-well plates, clear, PS
* SpectraMax M5 plate reader
* Reservoirs
* Adhesive sealing film for plate(s) during incubation

Reagents:

1. **PBS-T, pH 7.4**

For 1L buffer:

* 1. 1.44 g Na2HPO4
  2. 0.24 g KH 2PO 4
  3. 8 g NaCl
  4. 0.2 g KCl
  5. 1 L dH2O
  6. 500 μL Tween 20 (final conct.: 0.05%)

1. **TBS-T, pH 7.4**

For 1L of 10X TBS:

1. 24 g TRIS
2. 88 g NaCl
3. 1 L dH2O

For 1L of 1X TBS-T:

1. Dilute 100 mL 10X TBS with 900 mL dH20
2. 500 μL Tween 20 (final conct.: 0.05%)
3. **Binding Buffer**

25 mM HEPES, 40 mM KCl, 8 mM MgCl2, 100 mM NaCl, 1 mM DTT, 0.01% Tween pH 7.4

1. **5% Milk in TBS-T**
2. **Tau H-150 Santa Cruz 1:2000 in TBS-T**
3. **Goat anti-rabbit 1:2000 in TBS-T**
4. **TMB Substrate**
5. **1M HCl**

Procedure:

**Day 1**

1. Add 100μL of 0.005mg/mL EB3 (diluted with Ni-NTA elution buffer to match the buffer of concentrated EB3 stock) to appropriate wells of plates. For negative controls add Ni-NTA elution buffer without any protein to wells.
2. Cover plates and incubate at 37⁰C overnight.

**Day 2**

1. Discard protein from wells.
2. Wash 3X with PBS-T by adding 100 μL/well, incubating for 3 min on rocker, discarding PBS-T each time, and blotting the inverted plate on a paper towel to remove residual solution.
3. Add 30 μL of tau (dialyzed and diluted into Binding Buffer) to each well in triplicate, spanning a 16-dose 2-fold concentration gradient (100 μM to 0 μM). Each tau dose should be repeated in wells coated with buffer only as a control for nonspecific binding of tau to the plates.
4. Cover plates and incubate at room temperature on rocker for 3 hours.
5. Remove solutions and wash 3X with PBS-T.
6. Add 100uL of non-fat milk (5%) in TBS-T to all wells.
7. Incubate at room temperature for 5 min on bench.
8. Remove solution, but do not wash.
9. Add 50 μL primary antibody to all wells (rabbit anti-tau).
10. Incubate at room temperature for 1 hour on bench.
11. Remove solution and wash 3X with PBS-T.
12. Add 50 μL secondary antibody to all wells (goat anti-rabbit).
13. Incubate at room temperature for 1 hour on bench.
14. Remove solution and wash 3X with PBS-T.
15. Add 100 μL of TMB substrate to each well.
16. Incubate ~15 min covered in a dark place (bench drawer). This will yield a blue color.
17. Add 100 μL 1M HCl to each well to yield yellow color.
18. Read plate absorbance at 450nm on plate reader.