ELISA - Hsc70 affinity fo tau

Introduction

General ELISA protocol for measuring Hsc70 affinity for tau. From Zapporah Young.

Materials

- Plate
  - Fisherbrand, Flate bottom 96-well plates, clear, PS
- PBS pH 7.4 (for 1 L)
  - 1.44 g Na_2HPO_4
  - 0.24 g KH_2PO_4
  - 8 g NaCl
  - 0.2 g KCl
  - 1 L dH_2O
- PBS-T (for 1 L) - keep at 4°C
  - 1 L PBS pH 7.4
  - 500 μL Tween 20 (final conct.: 0.05%)
- 10X TBS pH 7.4 (for 1 L)
  - 24 g TRIS
  - 88 g NaCl
  - 1 L dH2O
- TBS-T (for 1 L) - keep at 4°C
  - 1 L TBS pH 7.4
  - 500 μL Tween 20 (final conct.: 0.05%)
- Binding buffer
  - 25 mM HEPES
  - 40 mM KCl
  - 8 mM $MgCl_2$
  - 100 mM NaCl
  - 0.01% Tween pH 7.4
- Immobilization Buffer (prepare freshly for each experiment)
  - 50 mM MES pH 5.5
  - 0.5 mM DTT
  - 1 mM ADP
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Procedure

Day 1

1. Add 30μL of 0.4mg/ml Hsp72/Hsc70 **(1μM)** (diluted with MES buffer pH 5.5 + fresh 0.5mM DTT + fresh 1mM nucleotide) to appropriate wells of plates. For negative control add MES buffer without any protein to wells.

2. Cover plates and incubate at 37°C overnight.

Day 2

3. Discard protein from wells

4. Wash with PBS-T 3x

   General wash procedure: 100 μL/well, incubate for 3 min on rocker, discard PBS-T each time, and blot the inverted plate on a paper towel to remove residual solution.

5. Add solutions of binding protein (4R0N, Tau, or other [Dilution series 100 μM -> 0 μM]) in 30 μL of BB (plus fresh 0.5 mM DTT and fresh 1 mM nucleotide) at appropriate concentrations.

6. Cover plates and incubate @ room temperature on rocker for 3 hours.

7. Remove solutions and wash 3x PBS-T

8. Add 100 μL of non-fat milk (5%) in TBS-T to all wells.

9. Incubate @ room temperature for 5 min on bench

10. Remove solution; do not wash.

11. Add 50 μL primary antibody to all wells Rb tau (tau H-150 Santa Cruz 1:2000 in TBS-T)

12. Incubate @ room temperature for 1 hour on bench.

13. Remove solution and wash 3x PBS-T.

14. Add 50 μL secondary antibody to all wells (goat anti-rabbit 1:2000 in TBS-T)

    *Note titers may be able to be decreased once maximum signal equilibrium is determined.

15. Incubate @ room temperature for 1 hour on bench.

16. Remove solution and wash 3x PBS-T

17. Add 100 μL of TMB substrate to each well.

    TMB substrate is light sensitive, and it is better to cap the bottle immediately after use. Also, if the plate is put in the dark during incubation, the signal will be increased to a large extent.

18. Incubate ~10-30 min or until blue

    *Incubation time may change depending on maximum signal/equilibrium determination.

19. Add 100μL stop solution (1M HCl) to yield yellow color.

20. Read @ OD_{450} on plate reader

    If reading >3.0 then reader's threshold has been exceeded. Ideally want values <1.8.