

COMPETITION ELISA

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Day 1

- Coat wells of at least four (4) 96-well plates with 100 μ l of 1 μ g/ml native tailspike in PBS; leave Column 1 and Rows A and H blank
- Incubate plates @ RT for 3 hr, or store at 4°C for up to two months (wrap to prevent dehydration)
- Dilute 1-50 μ l of fraction* to 250 μ l with PBS+TWEEN; mix with 250 μ l of .08 μ g/ml 1° Ab (for low-Kd antibodies) in glass fraction tubes. Also do native tailspike dilution series for standard curve: 0.625 – 0.0391 μ g/ml tailspike, 250 μ l, each mixed with 250 μ l 1° Ab. Also do 1° Ab positive controls (2 per plate), mixed with 250 μ l PBS+TWEEN. For all, seal, wrap and store overnight @ 4°C.
- *Deciding how much fraction to use: Because there will be much more tailspike at the top of the gradient (from native and other released chains), it is necessary to use far less fraction volume to stay within the reliable region of the tailspike standard curve. For fractions 1-12, I usually use 1 μ l fraction, and then for fractions 9-30, 15 μ l. More fraction may be appropriate for smaller ribosome preps (i.e., lower A_{260} values). For conversion of % Recognition to [Native Tspk Ag], it is really necessary to use % Rec. values below 70% (below 60% is ideal).

Day 2

- Wash plate wells with PBS+TWEEN, 3x
- Aliquot 100 μ l of mixtures into four wells of one column: three coated wells and one uncoated control (for example, Column 2 Row A-D); repeat for all samples, standards and blanks. DO NOT put sample in Column 1; save as blank. NOTE: Use timer to regulate rate of sample addition, starting a new sample every 20 sec.
- Incubate 30 min @ RT; aspirate out samples at same rate as sample addition (starting new sample every 20 sec); when not aspirating, refill emptied wells with 200 μ l PBS+TWEEN, using distri-man
- Aliquot 100 μ l 2° Ab (1:2000 dilution of goat anti-mouse alkaline phosphatase) into each sample well (do not fill Column 1)
- Incubate 30 min @ RT; wash wells 3x
- Aliquot 100 μ l PNPP substrate solution into each well; incubate until bright yellow (~60 min; check color development with plate reader)
- Read A_{405} of each plate

REAGENTS

- 10x PBS: 80g NaCl, 2 g KH_2PO_4 , 11.1 g Na_2HPO_4 (anhydrous), 2 g KCl, to 1 L w/ H_2O ; dilute 1/10 to use
- PBS+TWEEN: PBS plus 0.05% Tween-20 (from a 10% stock solution)
- PNPP substrate solution: 1 M ethanolamine, 1 mM MgSO_4 , to pH 9.8 with HCl; use 2.5 ml of this to dissolve each 5 mg tablet of PNPP