Screening group in Protein Chemistry Laboratory/NCI-Frederick

Robert J. Fisher, Head PCL

Criteria for NCp7 Small Molecule Screen

- Novel high affinity, zinc finger dependent, nucleic acid binding activity of NCp7 provides an assay for a new class of inhibitors
- The objective of this work was to find reversible inhibitors of the high affinity interaction between NCp7 and nucleic acid

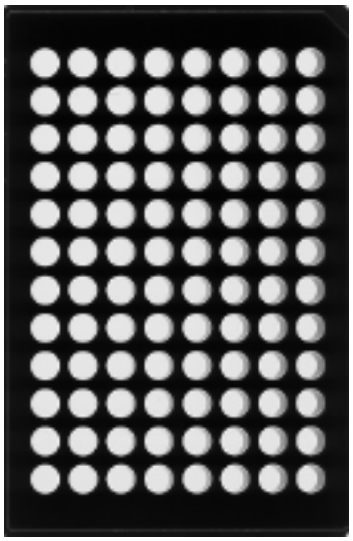
P7 screening assay
(Andy Stephen 2-01)

Bind p7 to plate (costar high-bind polystyrene Cat# 3631 or 3601)
Dilute p7 (200-1000nM) in 1xPBS/5mM b-mercaptoethanol
Add 100ml to each well, allow to sit overnight at 4^0C.
Add 200ml of 2% BSA in 1xPBS/5mM b-mercaptoethanol, incubate at room temperature for 1-2hours.
Aspirate off and wash with 2 x 200ml 1xPBS/5mM b-mercaptoethanol.
The plates can be stored at -20^0C, but more reproducible results are obtained if the plates are made fresh

Bind oligo to p7
Dilute oligo (concentration depends on amount of p7 immobilized, approx 1mM) in 1xPBS/100mM b-mercaptoethanol/0.05% Tween/5-10% DMSO.
Bind at room temperature for 1 hour, wash with 3 x 200ml 1xPBS/0.05% Tween

Detection.
Add 100ml 1:20000 dilution of nutravidin-HRP (stock 0.8mg/ml, from Pierce)
Incubate at room temperature for 1 hour, wash with 3 x 200ml 1xPBS/0.05% Tween
Add 100ml “supersignal” from Pierce and count in Wallac plate reader.

The NCp7 primary assay



- **p7NC immobilized to plate**
- **Block with BSA**
- **Wash, store at -20°C until use**
- **Add oligo (biotin-labeled) ± 10uM cpd**
- **Incubate for one hour**
- **Wash**
- **Incubate with streptavidin HRP**
- **Wash**
- **Develop**
- **read on Wallac Victor**
- **“hit threshold”=100% inhibition (yes/no)**
- **8 positive, 8 negative control/plate**
- **background ~1% false positives**