CTD oligopeptide Binding Assay using Magnetic Beads

Used in: Kim *et al* (2004) *Nature* **432:**517

(1) Oligopeptide Binding to Magnetic Beads

1. Take up 50 μl of Magnetic Beads (Dynal M280, 10 mg/ml) per binding reaction and transfer it into 0.6 ml tube.
   NOTE 1: Use Dynal beads, the cheaper ones have hideous backgrounds

2. Wash the beads twice with ice-cold oligopeptide binding buffer using magnetic stand.

   Oligopeptide Binding buffer (as described in JBC 276:28075):

25 mM	Tris-Cl (pH 7.6)
50 mM	NaCl
1 mM	DTT
5 %	Glycerol
0.03 %	Triton X-100

3. Resuspend in 500 μl of oligopeptide binding buffer and add 5 μg of oligopeptide.
4. Incubate with rotation for 1 hr at 4°C.
5. Spin down quickly and put the tube on magnetic stand.
6. Remove the supernatant and wash out the unbound oligopeptide with 500 μl of ice-cold oligopeptide binding buffer. Repeat washing twice.
7. Resuspend the beads with pre-cleared extracts.

(2) Pre-Clearing the extracts with Magnetic Beads

1. Take up 50 μl of magnetic beads and wash twice with 500 μl of ice-cold incubation buffer A or B, depending the buffer used in extracts preparation step.

   Incubation buffer A

10 mM	K-phosphate (pH7.7)
100 mM	KoAC
20 mM	MgoAC
5 mM	EGTA
10 %	Glycerol
0.1 %	NP-40
0.05 %	Triton X-100

   Incubation buffer B

50 mM	HEPES (pH 7.6)
50 mM	KoAC
10 mM	MgoAC
1 mM	EDTA
10 %	Glycerol
0.1 %	NP-40
0.05 %	Triron X-100

   plus 1 mM DTT and protease & phosphatase inhibitors
2. Add protein extracts (500 μg ~ 1 mg) to the pre-washed beads. Adjust the volume to 500 μl with incubation buffer, if necessary.
3. Incubate the tube with rotation for 1 hr at 4°C.
4. Centrifuge the tube at 14,000 rpm for 10 min at 4°C and transfer the supernatant (pre-cleared extracts) to the oligopeptide-bound magnetic beads [step 7 in section (1)]
5. Incubate the mixture with rotation at 4°C, O/N

(3) Wash & Elute bound proteins

1. Wash the mixture 3-5 times with 500 μl of ice-cold incubation buffer.
2. You can add 20-40 μl of 1x SDS-loading buffer directly to the beads for immediate PAGE analysis. But if necessary, elute the bound proteins with incubation buffer containing 0.5 M, 1.0 M, or 1.5 M KoAC;

   (i) resuspend the beads with 20-30 μl of 0.5 M KoAC-incubation buffer and put the tube on magnetic stand. Take the supernatant as an eluate. Repeat with 1.0 M or 1.5 M KoAC-incubation buffer.
   (ii) dialyze the salt eluate against incubation buffer for 2 hr at 4°C.
   (iii) add 1x SDS-loading buffer and analyze the sample through SDS-PAGE.