

## Histone H1 Kinase Assay

*The following procedure is routinely used to assay immunoprecipitated or pulled-down cyclin-dependent kinase (CDK) activities from maize endosperm extracts. EGTA is used on the assay buffer to minimize activity of CDPKs, which efficiently use histone H1 as substrate.*

Mix the reaction on an ice-chilled tube:

<b>Component</b>	<b>Volumes for 1 reaction (10 <math>\mu</math>l)</b>
Kinase Buffer	<b>7 <math>\mu</math>l</b>
histone H1	<b>1 <math>\mu</math>l</b> from 2.5 $\mu$ g/ $\mu$ l stock
ATP (pH 7.5)	<b>1 <math>\mu</math>l</b> from 4 mM stock
[gamma <sup>32</sup> P] ATP	<b>1 <math>\mu</math>l</b> from 10 $\mu$ Ci/ $\mu$ l stock

On ice, add 10  $\mu$ l of above mix to 10-20  $\mu$ l of beads (containing pulled-down or immunoprecipitated kinases) previously equilibrated on Kinase Buffer.

Resuspend beads by gently tapping the bottom of the tube.

Incubate at room temperature for 30 min.

Stop reaction by adding 5  $\mu$ l of 5x SDS sample buffer.

Boil samples for 5 min.

Separate samples on 12.5 or 15%, 1-mm thick SDS-PAGE mini-gels.

Let free [gamma<sup>32</sup>P] ATP run out of gel (roughly co-migrating with Bromophenol Blue); histone H1 migrates at around 30 kD.

If required, briefly stain gel by incubating it on Coomassie Blue R staining solution (10-15 min.) with gentle rocking, followed by destaining solution (three times, 5 min. each). Otherwise, just skip this step.

Incubate gel twice for 5-10 min. on 20% methanol, 10% glycerol.

Transfer gel to Whattman 3MM paper.

Move it to gel drier, gel facing up.

Cover with Saran Wrap, and apply vacuum for 40 min. at 50°C.

Expose dried gel to Phosphorscreen or X-ray film for at least 5 h.

## REQUIRED REAGENTS AND SOLUTIONS

### Kinase Buffer

50 mM Tris-Cl pH 7.5  
20 mM EGTA  
10 mM MgCl<sub>2</sub>  
1 mM DTT  
1 mM beta-glycerolphosphate  
Make 1-ml aliquots and store at -20°C.

**histone H1 (2.5 mg/ml)**      Dissolve 2.5 mg of Histone H1 (Sigma Type III) in 1 ml of 25 mM Tris-HCl pH 8.0.  
Make 20- $\mu$ l aliquots and store at -20°C.

**ATP (100 mM):**      Dissolve 60 mg of ATP (disodium salt) in 0.8 ml of 0.5M Tris-Cl pH 7.5  
Adjust volume to 1 ml, make 10- $\mu$ l aliquots and store at -80°C.  
Alternatively, further dilute to 10 mM, make 10- $\mu$ l aliquots and store at -80°C. Make 4 mM working solutions fresh. Do not freeze and thaw ATP solutions repeatedly.

**[gamma-<sup>32</sup>P]ATP Amersham #AA0068**      10 mCi/ml (10  $\mu$ Ci/ $\mu$ l)