

Kinase Assay

1. Prepare Buffers-

H1 Kinase Buffer (can be stored):

20 mM HEPES (pH7.7)

50 mM NaCl

10 mM MgCl₂

2 mM EDTA

0.02% Triton-X 100

Kinase Reaction Master Mix:

4.5 μ l 1mM ATP

1.2 μ l 5mCi/ml γ -³²P-ATP (from Cobb Lab)

95 μ l Kinase Buffer

2. Aliquot Kinase, substrate and Master Mix into 20 μ l reaction. (For phosphatase assays, make master kinase reaction and aliquot after IP -> p236: 80 μ l CDH1 fragment, 40 μ l GST-cdc2/cyclin B1 kinase from purified Sf9 (1:100 Dilution), 200 μ l Master Mix for 20 reactions)

Incubate 1 hour at room temperature.

3. For ppase assay, add 5 μ l solid GST-beads and incubate 30 minutes at room temperature, shaking.

4. Aliquot kinase reaction into 20 μ l reactions, add CDC14 (amount varies according to prep). If kinase assay alone, quench with SB, run gel.

Incubate 1 hour at room temperature.

5. Stop reaction with Sample Buffer, run 15% gel at 100V for 2 hours on CDH1 Fragment.