

APC Activity Assay

1. Prepare Buffer:

XB Buffer: 100 ml 20X XB Salt
 20 ml 1M HEPES (pH7.7)
 34.2g Sucrose
 Per 2 Liters

XB High Salt Buffer:

XB Buffer
400 mM KCl
0.5% NP40

2. Immunoprecipitate APC from interphase extract:

 400 μ l Interphase Extract
+ 40 μ l Solid anti-**APC3** Beads (Wash beads 2X with XB)

 Incubate at 4°C for > 2 hours, rotating

3. Wash Beads 4X with High Salt XB Buffer

Wash Beads 2X with XB Buffer

Resuspend to 100 μ l total in XB

Aliquot 5 μ l 1:1 suspension of beads per reaction

4. Treat APC with various factors to activate, incubate shaking at room temperature for 1 hour.

5. Wash Beads 2X with XB Buffer

6. Do ubiquitination activity assay:

Master Mix 7.5 μ l Ubiquitin
 7.5 μ l Energy Mix
 3.0 μ l UBCx
 10 μ l Substrate (at proper dilution)
 15 μ l E1 enzyme
 15 μ l XB Buffer ~ 16 Reactions
Aliquot 3.0 μ l per Reaction

7. Incubate for 1 hour, room temperature, shaking
8. Quench with 40 μ l Sample Buffer
9. Load 15 μ l onto 12.5% gel, run 2 hours at 140V.
10. Transfer to membrane and western blot with anti-myc antibody