



CycLex Protocol P-005

Immunoprecipitation

Cell extraction and Pre-clearance

1. Suspend $\sim 1 \times 10^7$ cells (100mm dish subconfluent) into 1ml of E1A Buffer.
2. Vortex
3. Keep on ice for 30 min.
4. c.f.g. 15,000 rpm for 10 min.
5. Keep the sup.
6. Add 50-100 μ l of ProteinA Sepharose (50% suspension) into the extract (Pre-clearance step)
7. Rotate the tube for 1hour at 4°C
8. c.f.g. 15,000 rpm for 5 min.
- 9.Keep the sup.

Immunoprecipitation

1. Add IP antibody 2-4 μ g to 0.5 ml of cell extract.
2. Rotate the tube for 30min. at 4°C.
3. Add 20-40 μ l of ProteinA Sepharose (50% suspension) into the extract.

4. Rotate the tube for 1-4 hours at 4°C.
5. c.f.g. 12,000 rpm for 1min.
6. Remove the sup.
7. Wash the resin 4 times with E1A buffer.
8. c.f.g. 12,000 rpm for 1 min.
9. Add 20 ul of 2X SDS-Sample buffer to the resin.
10. Boil for 2min.
11. c.f.g. 15,000 rpm for 1 min.
12. SDS-PAGE
13. Western Blotting

E1A Buffer

50 mM Hepes pH 7.2~7.6

250 mM NaCl

0.2 % NP-40

5 mM EDTA-2Na (pH 7.5)

10 % Glycerol

0.5 mM DTT

protease inhibitor cocktail

1 µg/ml pepstatin, 1 µg/ml leupeptin, 0.3 µM aprotinin, 15 µM E-64

phosphatase inhibitor cocktail (in case of using phosphor-specific antibody)

2.5 mM Sodium pyrophosphate, 1 mM beta-Glycerolphosphate, 1 mM Na₃VO₄