FG winds

Issue 6
April, 2015

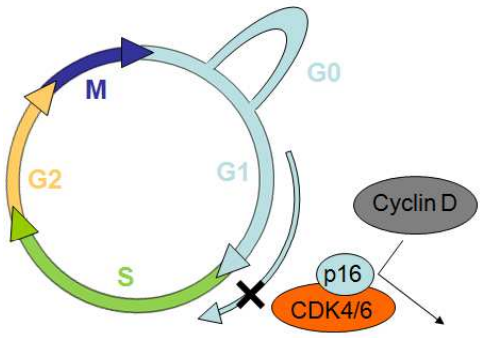
Immunoprecipitation (2)

Summary

Immnoprecipitation is an effective approach for the detection of a slight amount of endogenous protein in cells. This time, we performed immunoprecipitation of p16 of HeLa cell, by using Protein G beads.

p16 is known as one of inhibitors of cyclin-dependent kinase (CDK), and is also referred to as p16 INK4a because it belongs to INK4 family. The cell cycle is composed as shown in the right figure. CDK4/6 is activated by forming a complex with Cyclin D, phosphorylates target protein, and induces progress in the cell cycle from G1 phase to S phase. p16 works to stop the progress of the cell cycle by competitively inhibiting the complex formation of CDK4/6 and Cyclin D.

It is known that p16 gene functions as a tumor suppressor gene, and is mutated or deleted in a human cancer cell in most cases. When a cell receives oncogenic stress or signals, an expression of p16 gene increases, the function of CDK4/6 is inhibited, and the cell cycle progression is stopped . This is regarded as a self-defense mechanism to protect normal cell from canceration.



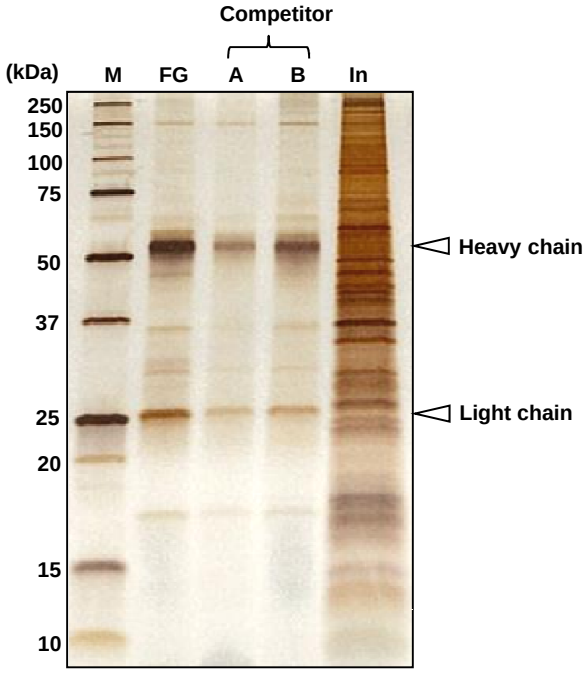
Inhibition pattern of cell cycle progression by p16

Result

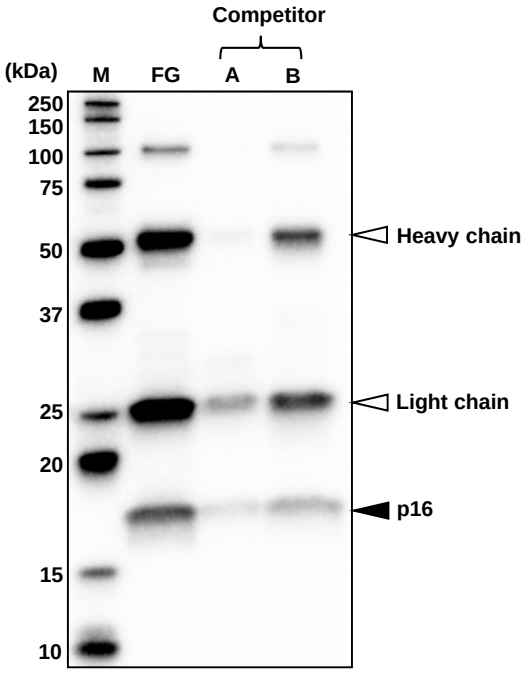
Using 0.1mg of Protein G beads from each company and 5ug of Anti-p16 antibody, we immunoprecipitated p16 from HeLa cell extracts, and performed silver staining and Western blotting after SDS-PAGE.

As the result of the Western blotting, we comfirmed that the p16 was purified and FG beads® achieved the largest recovery amount.

The result of the silver staining showed that the proteins interacting with the p16 were co-immunoprecipitated because plural clear bands were detected.



Silver staining



Western blotting

Materials and method

■Materials

1. Protein G beads
2. HeLa cell extracts (cytosolic fraction) – 3mg/ml
3. PBS(-) (137mM NaCl, 2.7mM KCl, 8.1mM $Na_2HPO_4 \cdot 12H_2O$, 1.5mM KH_2PO_4)
4. Wash Buffer A (10mM HEPES-NaOH(pH7.9), 50mM KCl, 0.2mM EDTA, 10%(v/v) glycerol)
5. Wash Buffer B (20mM HEPES-NaOH(pH7.9), 150mM KCl, 1mM $MgCl_2$, 0.2mM $CaCl_2$, 0.2mM EDTA, 10%(v/v) glycerol, 0.1% NP-40, 0.2mM PMSF)
6. SDS sample buffer (62.5mM Tris-HCl (pH6.8), 0.005% BPB, 2% SDS, 10% glycerol, 5% 2-mercapto ethanol)
7. Anti-p16 antibody (from Abcam)
8. Anti-mouse IgG, HRP-Linked Whole Ab Sheep(from GE healthcare)
9. Transfer buffer (25mM Tris, 192mM Glycine, 20%(v/v) Methanol)
10. Blocking buffer (from Thermo)
11. TBS-T buffer (20mM Tris-HCl (pH7.5), 500mM NaCl, 0.1% Tween20)

■FG beads® information

Product name	Protein G beads
Product number	TAS8848N1173
Storage temperature	2-8°C
Storage buffer	10mM HEPES(pH7.9), 50mM KCl, 1mM EDTA, 10%glycerol
Size of beads	190nm ± 20 nm
IgG binding capacity	>100ug mouse IgG /mg of beads

■Method 1 (Binding Antibody)

1. **Wash**
   Transfer 0.1mg of beads to a tube.
   Wash beads with 200ul PBS(-) 2 times at 4°C.
2. **Bind Antibody**
   Add antibody (5ug) diluted in 200ul PBS(-) to beads.
3. **Reaction**
   Mix for 30min at room temperature.
4. **Wash**
   Wash antibody binding beads with Wash buffer A 2 times at 4°C.

■Method 2 (Immunoprecipitation)

1. **Add sample solution**
   Add 200ul HeLa cell extracts .
2. **Reaction**
   Resuspend beads and incubate with rotation for 120min at 4°C.
3. **Wash**
   Separate magnetically and remove supernatant.
   Wash beads with Wash buffer B 3 times at 4°C.
   Separate magnetically and remove supernatant.
4. **Elution**
   Add 40ul SDS sample buffer and resuspend beads.
   Boil for 5min and remove the beads.
5. **Analyze the samples by SDS-PAGE and silver staining**

■Methods 3 (Western blotting)

1. Perform SDS-PAGE, and place the gel in Transfer buffer for about 10min.
2. Transfer the protein from the gel to a PVDF membrane.
3. Block the membrane with the Blocking buffer for 15min at room temperature.
4. Dilute the primary antibody with the Blocking buffer to 1/150.
5. Incubate for 60min at room temperature.
6. Wash the membrane with TBS-T buffer 3 times.
7. Dilute the secondary antibody with the TBS-T buffer to 1/2000.
8. incubate for 60min at room temperature.
9. Wash the membrane with TBS-T buffer 5 times.
10. Detect with a chemiluminescence substrate.