Protocol for immunoprecipitation of target protein from cell lysate

AV-06 Immunoprecipitation (IP)

1.0 Introduction / Description

This method is intended for the evaluation of the specificity of the antibody of interest in immunoprecipitation assay using the lysates generated in the protocol for induction and lysate production.

Lysates from cells treated with 1000ng/ml doxycycline and from cells treated with 50ng/ml doxycycline are used to determine the immunoprecipitation specificity of the antibody of interest compared to control normal mouse IgG and to FLAG-M2 antibodies.

2.0 Materials

Glassware/Plasticware/Instruments and tools

autoclaved 1.5 ml tubes- Axygen scientific cat.# 22-281
magnet holder (DynaMag™-2)- Invitrogen cat.# 12321D
rotator at 4°C
tube shaker
heat block at 70°C

Reagents

normal mouse IgG- Santa Cruz, cat.# 2025
anti FLAG-M2 antibody- SIGMA cat.# F1804
CDI mAb to be validated
Protein G-conjugated Dynabeads- Invitrogen cat.# 100.03D
NuPAGE® LDS sample buffer (Life technologies corp. cat.# NP007)
β-mercaptoethanol (SIGMA-Aldrich cat.# M6250)

3.0 Immunoprecipitation (IP)

- ✓ IP is carried out on 120μl of cell-lysate (corresponds to approx. 400,000 cells) obtained in Section 4. using either ant-FLAG M2 antibody, antibody of interest or normal mouse IgG control antibody.
- ✓ IP will setup in 96well deep-well plates using the following schema:

	1	2	3	4	5	6	7	8	9	10	11	12
Tube=>	C	B	C	C	C	B	C	C	C	B	C	C
Ab=>	IgG	CDI mAB		FLAG	IgG	CDI mAB		FLAG	IgG	CDI mAB		FLAG
A	Lysate Plate-1				Lysate Plate-9				Lysate Plate-17			
B	Lysate Plate-2				Lysate Plate-10				Lysate Plate-18			
C	Lysate Plate-3				Lysate Plate-11				Lysate Plate-19			
D	Lysate Plate-4				Lysate Plate-12				Lysate Plate-20			
E	Lysate Plate-5				Lysate Plate-13				Lysate Plate-21			
F	Lysate Plate-6				Lysate Plate-14				Lysate Plate-22			
G	Lysate Plate-7				Lysate Plate-15				Lysate Plate-23			
H	Lysate Plate-8				Lysate Plate-16				Lysate Plate-24			

- ✓ Lysate is incubated with antibody overnight at 4°C on a rotator.
- ✓ On Day2 prepare Protein G-conjugated Dynabeads:
    - Wash 20µl of Protein G-conjugated Dynabeads (DynaMag™-2; life technology) per IP in 100µl of lysis buffer
    - Remove supernatant using magnet holder (DynaMag™-2; Life Technology)
    - Repeat the wash a second time
- ✓ Resuspend the washed protein G-dynabeads in 50 µl of lysis buffer per IP

- ✓ Add 50 µl of protein-Dynabeads/lysis buffer to the lysate/antibody solution in each well of the 96deep-well plate
- ✓ Incubate for 2hrs on a rotator at 4°C
- ✓ Remove the supernatant containing the unbound proteins using the magnet holder
- ✓ Wash beads 3 times in 500µl of lysis buffer
- ✓ Resuspend beads in 30µl of 1X LDS sample buffer (Invitrogen) with 5% β-mercaptoethanol.
- ✓ Incubate at room temperature for 10 minutes on a plate-shaker.
- ✓ Heat at 90°C for 10 minutes
- ✓ Collect supernatant using the magnetic holder and transfer it into a 96well PCR plate.