

Immunoprecipitation (IP)

1. Resuspend cells in 1 ml of lysis buffer (1mM PMSF). Usually 1 ml of buffer per one 10 cm plate.
2. Keep cell supernatant on ice for several minutes.
3. Sonicate (setting number 5 for 10 seconds).
4. Transfer solution to an eppendorf tube.
5. Spin down at 10,000 rpm/2 minutes/ 4°C.
6. Remove supernatant.
7. Add 40-50µl of active Staph.
8. Spin down at 10,000 rpm/2 minutes/ 4°C.
9. Discard pellet, retain aqueous solution.
10. Aliquot appropriately (usually 100µl/reaction).
11. Add 100µl or monoclonal or 5µl of polyclonal antibody to each tube.
12. Incubate on ice 30-60 minutes.
13. Add 50 µl of Staph. to each tube.
14. Incubate on ice 15-20 minutes.
16. Spin down at 10,000 rpm/5 minutes.
17. Discard supernatant, save pellet.
18. Wash pellet 2-3 X with SNNTE.
19. Wash pellet 1 X with NTE.
20. Spin down at 10,000 rpm/5 minutes.
21. Resuspend pellet with 20µl of 2 X sample buffer.
22. Boil for 3 minutes.
23. Spin down at 13,000 rpm/5 minutes.
24. Save supernatant for running gel. Store at - 20°C.

Solutions and Activation of Staph cells:

Lysis Buffer: per 100 mls:

50 mM Tris pH 8.0.....	5 mls of 1M Tris pH 8.0
5mM EDTA.....	1 ml of 0.5 M EDTA
150mM NaCl.....	3 ml of 5M NaCl
0.5% NP-40.....	5 mls of 10% NP-40
ddH2O.....	86 mls

Protease inhibitors (available from BMB):

Always use:

- PMSF: (stock = 100mM in ETOH) use 10-40µg/ml of LB
- EDTA: (stock = 50 mg/ml) use 0.2-0.5 mg/ml of LB
- Leupeptin: (stock = 1 mg/ml) use 0.7 µg/ml of LB

Recommended:

- Pepstatin: (stock = 0.5 mg/ml) use 0.7µg/ml of LB
- Aprotinin: (stock = 5 mg/ml) use 2-10 µg/ml of LB

PMSF: 100mM PMSF (TOXIC!!!!) in 95% EtOH; store at -20°C

TENN: per 100 mls:

50mM Tris pH 7.4.....	5 mls of 1M Tris pH 7.4
5mM EDTA.....	1 ml of 0.5M EDTA
0.5% NP-40.....	5 ml of 10% NP-40
150mM NaCl.....	3 mls of 5M NaCl
ddH ₂ O.....	86 mls

SNNTE: per 100 mls:

5% sucrose.....	20 mls of 25% sucrose
50mM Tris pH 7.4.....	5 mls of 1M Tris pH 7.4
5mM EDTA.....	1 ml of 0.5M EDTA
0.1% NP-40.....	1 ml of 10% NP-40
0.5mM NaCl.....	10 mls of 5M NaCl
ddH ₂ O.....	63mls

NTE: per 100 mls:

50mM NaCl.....	1ml of 5M NaCl
10mM Tris pH 7.4.....	1 ML of Tris pH 7.4
1mM EDTA.....	0.5 mls of 0.5M EDTA
ddH ₂ O.....	97.5 mls

Sample Buffer (2x):

10% glycerol
2% SDS
0.02% Bromophenol blue
10% β-mercaptoethanol
0.125M Tris pH 6.8
Filter sterilize.

Staph. aureus cells: 10% cell suspension in ddH₂O or PBS/Azide (better); aliquot and store at -20°C. Stable 4-6 months. (Kessler, J. Immunology 117 (1976) : 1482)

Activation of Staph: (use freshly activated Staph for each IP time)

1. Thaw cells at room temperature
2. Spin down in microfuge for two minutes (mark tube where minicus is)
3. Discard supernatant
4. Wash cell pellet with TENN. Resuspend cells in equal original volume of TENN.
5. Vortex.