How to prep Staph

Calbiochem: Pansorbin Cells. Cat. No. 507858

1. Vortex/pipet vigorously to get staph into solution. Put into centrifuge tubes and spin down gently (but make sure it is pelleted thoroughly)

2. Decant supernatant and resuspend in 1X RIPA. Wash 3X.

3. Resuspend in 50 ml RIPA + 3% SDS + 5% BME (final concentrations) and boil for 30 minutes.

4. Spin down and wash 3X in RIPA buffer

5. At this point, all black stuff should be gone and staph should resuspend easily. If not repeat steps 3 and 4.

6. Resuspend in RIPA → 25g staph/250 ml RIPA. Aliquot into eppendorfs and store at -20°C

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Staph

- is frozen at -20°C in 1 ml aliquots (in RIPA buffer)
- before use:
  - spin down + wash 3X in 1X NP-40 lysis buffer
  - this will take some work to resuspend

- Staph in 1X NP-40 lysis can be stored 4°C for 1 month
- pool to minimize variability if

2X M Staph

- wash Staph as above
- to 1 ml resuspended Staph, add 50x RmM IgGl (Coppell)
- rotate 30'
- wash 2X 1X NP-40 lysis + resuspend finally in 1X NP-40 pool different aliquots to minimize variability
- store 4°C up to 1 month