

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 -20C

Staph

- is frozen at -20° 1ml aliquots (in RIPA buffer)
- before use on IP's:
 - spin down + wash 3X in 1X NP40 lysis buffer
 - * this will take some work to resuspend
- staph in 1X NP40 lysis can be stored 4° for 1-2 months
- * pool + to minimize variability

R&M Staph

- wash staph as above
- to 1ml resuspended staph, add 50 μ l R&M IgG (Cappel)
- rotate 30'
- wash 2X \pm 1X NP40 lysis + resuspend finally in 1ml 1X NP40
- pool different aliquots to minimize variability + store 4° up to ~ 1 month