TCA protein extraction method

We use this method (which we did not invent) for quick protein preps to use for western blots. We have found that it does not work well for reproducibly precipitating histones (so don't use those for your loading control if you use this method), but for other proteins we have evaluated it seems to work well.

1. Pellet 2.5 ml cell culture (10^8 cells/ml, if your culture is less dense, use more)
2. Wash one time with H_2O
3. Re-suspend pellet in 0.25 ml ice cold 16.6% TCA, then add ~ 500 ul ice cold glass beads (we use 0.5 mm zirconia/silica beads, Biospec Products).
4. Vortex 5min in the Bullet Blue Blender at speed 8 (4^oC) – this agitates cells more than standard vortexing – if you are vortexing, you'll have to go longer. Remove lysate to a clean microfuge centrifuge tube and place on ice.
5. Wash beads 3 x 0.3ml 5% TCA and add to the lysate.
   (Alternative method: Invert tube, puncture bottom of tube with red hot 25G needle. place tube into a fresh tube and collect lysate by spinning at 3000rpm for 2min. Place on ice.)
6. Spin 5 min, at top speed (13000 rpm) in a precooled (4^oC) rotor
7. Aspirate and discard supernatant. Wash the pellet with 0.5 ml 100% EtOH (ice cold)
8. Spin 2 min, top speed at 4^oC
9. Remove supernatant and dry the pellet
10. Re-suspend in 25 ul of 2x SDS-PAGE sample buffer then vortex. Add 1M Tris base until the dye turns blue (usually ~ 2ul). Add 5 ul of 1M DTT and then enough H_2O to bring the volume to 50ul. Vortex.
11. Heat at 90^oC for 10 min, quickly spin down and transfer the supernatant to a clean tube.
12. The sample is ready to use for western blotting. In our experience, loading about about 5 – 10 ul of sample per lane is sufficient is sufficient for detection of candidate proteins by western blotting.

To make 16.6% TCA

1. Use Maniatis Protocols manual recipe that is 500 g TCA plus 227 g of water then dilute to 16.6%.
2. Purchase TCA solution (6.1N), which ~100%, then dilute to 16.6%