TCA Protein Extracts

TCA is caustic – wear gloves and eyeprotection

1. Grow cells to an OD of 0.2.
2. Spin down 100 ml of cells @3000 rpm for 5 minutes (depending on protein being analyzed, can use a smaller volume of cells here).
3. Resuspend in 1ml of 1.2 M Sorbitol and transfer to a 2ml Eppendorf.
4. Spin down at full speed for 30 seconds and discard supernatant. **
5. Add 100ul of 20% TCA and vortex briefly to resuspend cells.
6. Add *micro* glass beads to the 0.5 ml mark of the Eppendorf.
7. Vortex using genie2 for 5 minutes @ 4°C.
8. Let stand for one minute@ 4°C.
9. Vortex again for 1 minutes @ 4°C.
10. Add 900 ul of 5% TCA and vortex briefly.
11. Remove 800 ul of the extract to a fresh Eppendorf.
12. Centrifuge for 10 minutes @3000rpm. Discard supernatant to leave the protein pellet.***
13. Add 250 ul of 1x Laemmli (25mM Tris) and vortex briefly to resuspend. If solution turns yellow add more Tris base (1M) – 10 ul is usually enough.
14. Boil for 3 minutes.
15. Spin down @3000 rpm for 10 minutes. (in fact short high speed spin should be ok here).
16. Transfer supernatant to a fresh Eppendorf.
17. Load 5-30 ul per lane. Keep extracts frozen at -20°C.

**At this point you can freeze the samples on dry ice and process them when ready.
*** samples can also be stored at this stage at -20°C.

It may be important to spin the extract at 3000 rpm as opposed to 14000 rpm.

NB. 100% TCA is 500g TCA and 227ml water.

1x laemmli buffer (inc for TCA)

	standard 1x laemmli	higher tris for tca extracts	final concentration
H20	2.7 ml	2.2 ml	
1M Tris HCl pH 6.8	0.5 ml	1 ml	62.5 mM (125 mM)
glycerol	0.8 ml	0.8 ml	10%
10% SDS	1.6 ml	1.6 ml	2%
betamercaptoethanol	0.4 ml	0.4 ml	5%
BPB	2 ml of 0.1% BPB	2 ml of 0.1% BPB	
	8 ml total		

Add more 1M Tris base during TCA extraction if solution goes yellow (10 ul per sample is usually enough).

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