HOST-PATHOGEN INTERACTIONS LAB
Benjamin Aroeti, Ph.D.
aroeti@mail.huji.ac.il

TITLE: TCA PRECIPITATION

Date:Thursday, February 10, 2022

NAME: MARIO LEBENDIKER

TCA Precipitation – Mario Lebendiker

TCA-DOC

For precipitation of very low protein concentration

1) To one volume of protein solution, add 1/100 vol. of 2% DOC (Na deoxycholate, detergent).
2) Vortex and let sit for 30min at 4ºC.
3) Add 1/10 of Trichloroacetic acid (TCA) 100% vortex and let sit ON at 4ºC (preparation of 100% TCA: 454ml H2O/kg TCA. Maintain in dark bottleat 4ºC.Be careful, use gloves!!!).
4) Spin 15min 4ºC in microfuge at maximum speed (15000g). Carefully discharge supernatant and retain the pellet: dry tube by inversion on tissue paper (pellet may be difficult to see). *[OPTION: Wash pellet twice with one volume of cold acetone (acetone keep at –20ºC). Vortex and repellet samples 5min at full speed between washes].*
5) Dry samples under vaccum (speed vac) or dry air. For PAGE-SDS, resuspend samples in a minimal volume of sample buffer. (The presence of some TCA can give a yellow colour as a consequence of the acidification of the sample buffer ; titrate with 1N NaOH or 1M TrisHCl pH8.5 to obtain the normal blue sample buffer colour.)

Normal TCA

To eliminate TCA soluble interferences and protein concentration

1) To a sample of protein solution add Trichloroacetic acid (TCA) 100% to get 13% final concentration. Mix and keep 5min –20ºC and then 15min 4ºC; or longer time at 4ºC without the –20ºC step for lower protein concentration. Suggestion: leave ON if the protein concentration is very low.
(preparation of 100% TCA: 454ml H2O/kg TCA. Maintain in dark bottleat 4ºC.Be careful, use gloves!!!).
2) Spin 15min 4ºC in microfuge at maximum speed (15000g). Carefully discharge supernatant and retain the pellet: dry tube by inversion on tissue paper (pellet may be difficult to see).
3) For PAGE-SDS, resuspend samples in a minimal volume of sample buffer. (The presence of some TCA can give a yellow colour as a consequence of the acidification of the sample buffer ; titrate with 1N NaOH or 1M TrisHCl pH8.5 to obtain the normal blue sample buffer colour.)