

ICAT Labeling Procedure ('Old ICAT')

1. Dissolve protein in 5mM Tris pH 8.3, 10mM EDTA and boil protein sample for approximately 5 minutes, then chill over ice for approximately 20 minutes.
2. Add reducing agent (DTT, TBP, or TCEP) to a concentration of 5mM. Incubate at 37°C for 20 minutes. If using DTT or TBP as a reducing agent, precipitate the proteins (to remove DTT) using cold acetone. Add 6-7 volumes of acetone stored at -20°C. Allow the protein to precipitate out of solution. Spin the sample, collect, dry the pellet, and resuspend in a minimal amount of 5mM Tris pH 8.3, 10mM EDTA . If using TCEP, cold acetone precipitation is optional.
3. Dissolve the ICAT in 20uL of acetonitrile per tube. Add the sample to the ICAT tube in a minimum concentration of 6-fold molar excess ICAT. This typically corresponds to 1 tube for every 200ug of protein labeled. It is important that the ICAT reaction is conducted in as small a volume as possible—ideally less than 150ul per tube or the ICAT concentration will be too low to allow adequate labeling.
4. Incubate the reaction at room temperature in the dark (put in a box covered with foil) for a minimum 2 hours. Agitation/gentle shaking is typically done, though it is not necessary.
5. Check ICAT labeling as a shift in the protein bands on SDS-PAGE.
6. The reaction can be quenched by adding 5mM DTT and incubating at 55°C for 20 minutes.
7. Combine ICAT labeled samples to the desired ratio.

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