

Tips and tricks of the trade

Two Precipitants are Better than One

Sometimes, even slight modifications are sufficient to significantly improve established protocols.

Lab Hint

The guanidinium-thiocyanate-phenol-chloroform (GTPC) extraction protocol (also called Trizol-extraction), introduced by Chomczynski and Sacchi in 1987, is applied by many researchers to isolate both nucleic acids and proteins. It is based on the different solubilities of RNA, DNA and protein molecules in water and organic solvents.

Samples are usually mixed in Eppendorf tubes with the GTPC-reagent, and centrifuged to separate organic and aqueous phases. Proteins accumulate in the lower phenol-chloroform phase, while DNA stays in the interphase and RNA goes to the upper phase. Upper phase and interphase are withdrawn with a pipette, whilst RNA and DNA are precipitated with isopropanol and ethanol, respectively. Proteins in the remaining organic phase may then be precipitated with isopropanol.

GTPC extraction produces high amounts of DNA and RNA, however, protein isolation is time-consuming and yields are low (about 60%). To get better protein yields Soroth Chey and his colleagues at the Institute for Virology in Leipzig slightly modified the protein isolation step of the GTPC protocol by adding ethanol and bromo-chloropropane to precipitate proteins (Chey *et al.*, *Anal. Biochem.*, 2011, 411:164-6). The modified protocol works well for proteins extracted from cells or tissues and yields up to 95% of protein.

The main steps are as follows:

▶ Add 2.5 volumes of lab grade ethanol (750 µl) to one volume (300 µl) of protein solution (phenol-phase) and vortex for 15 seconds.



The various pipetting steps during GTPC extraction require a steady hand.

- ▶ Add 200 µl of bromo-chloropropane to the suspension and vortex again.
- ▶ Pipette 600 µl of bidistilled water to the sample and mix by vortexing.
- ▶ Separate the phases by centrifugation at 12,000 g for five minutes.
- ▶ Remove the upper aqueous phase (proteins rest in the interphase) and add 1 ml of lab grade ethanol to the remaining organic phase. Vortex for a few seconds and then centrifuge the mixture at 12,000 g for five minutes.
- ▶ Withdraw the ethanol supernatant and wash the pellet once with 1 ml ethanol, vortex briefly and centrifuge at 12,000 g for five minutes.

- ▶ Air-dry the protein-pellet or dry in a slight vacuum.

Solve the protein-pellet by pipetting it up and down in a 4% SDS solution. You may also heat the sample to 55°C - 60°C to get the proteins into solution. The proteins are ready for immediate use or may be stored in the freezer at -20°C.

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Do you have any useful tips?

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