

High-Yield Skeletal Muscle Protein Recovery from TRIzol® after RNA and DNA Extraction

Yuan Wen¹, Ivan Vechetti¹, Taylor Valentino¹, John J McCarthy¹

¹University of Kentucky

1 Works for me dx.doi.org/10.17504/protocols.io.bii3kcg9



Yuan Wen

ABSTRACT

Extraction of DNA, RNA, and protein from the same sample would allow for direct comparison of genomic, transcriptomic, and proteomic information. Commercially available kits exhibit poor protein yield and TRIzol® reagent produces a protein pellet that is extremely difficult to solubilize. In response to these limitations, this study presents an optimized method for the extraction of protein from the organic phase of TRIzol® reagent that allows for higher yield recovery of skeletal muscle protein compared to direct homogenization in a common protein lysis buffer. The presented method is inexpensive, simple and fast; requires no additional treatment of the protein pellet for dissolution; and is compatible with downstream western blot applications.

DOI

dx.doi.org/10.17504/protocols.io.bii3kcg9

PROTOCOL CITATION

Yuan Wen, Ivan Vechetti, Taylor Valentino, John J McCarthy 2020. High-Yield Skeletal Muscle Protein Recovery from TRIzol® after RNA and DNA Extraction. **protocols.io**
dx.doi.org/10.17504/protocols.io.bii3kcg9

LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jul 13, 2020

LAST MODIFIED

Jul 21, 2020

PROTOCOL INTEGER ID

39227

MATERIALS TEXT

Recipes

SDS-Urea Buffer

100 mM Tris (pH 6.8)

12% glycerol

4% SDS

0.008% bromophenol blue

2% β-mercaptoethanol

5 M Urea

*Add Halt™ Protease Inhibitor Cocktail (ThermoFisher 78430) immediately before use

RIPA Buffer

50 mM Tris-HCL pH 7.4

1% Triton-X100

0.5% Sodium deoxycholate

0.1% SDS
150 mM NaCl
2 mM EDTA
50 mM NaF
*Add Halt™ Protease Inhibitor Cocktail (ThermoFisher 78430) immediately before use


Reagents

- TRIzol® (Invitrogen, cat. no. 15596-026) – **Caution:** Toxic and corrosive.
- 1-Bromo-3-chloropropane (BCP, Sigma, cat.no. B62404) - less toxic substitute for chloroform [1]
- Ethanol (EtOH)

Equipment

- Bullet Blender® Tissue Homogenizer (Next Advance)
- Vertical gel system (protean II Bio-Rad system with gel plates 16 x 20 cm; Bio-Rad)
- Tabletop temperature controlled centrifuge

RNA Extraction

1 

Mince muscle tissue into small (~10 mg) pieces.

- **Critical step:** The large amount of sarcomeric proteins in skeletal muscle makes it difficult to solubilize; starting with small masses of muscle initially will increase protein yield.

2 Isolate RNA using TRIzol® reagent, following manufacturer's instructions.

- **Important point:** Use 0.5 ml of TRIzol® reagent per piece (<15 mg) of tissue.
- **Important point:** For better phase separation, use 100 µl of BCP instead of Chloroform.

3 Remove top RNA fraction (aqueous phase) according to manufacturer's recommendation.

4 Follow manufacturer's instructions for RNA precipitation, wash, and re-suspension or choice of RNA column purification kit.

5 Proceed to DNA precipitation.

DNA Precipitation

6 

Precipitate DNA following TRIzol® manufacturer's instructions.

- **Critical Step:** Use 150 µl of 100% EtOH and centrifuge at 5000g for 10 minutes.


7 Wash and re-suspend DNA pellet according to manufacturer's instructions or choice of genomic DNA column purification kit.

8 Proceed to Protein Recovery.


Protein Recovery

9 Transfer an aliquot (~0.45 ml) of protein-TRIzol®-EtOH solution into 2 ml tube.

- **Important point:** This type of tube is required due to large volume (~1.8 ml).

- 10 Add 650 µl of 100% EtOH (protein precipitation step) to protein-containing phenol–ethanol supernatant.
- 11 After vortexing the samples, mix with 100 µl of BCP and vortex again.
- 12 For phase separation, add 600 µl water and vortex vigorously.
- 13 Centrifuge at 12,000g for 5 minutes at room temperature.
- 14 Remove and discard the upper aqueous layer (proteins are in the interphase).
- 15 Add 700 µl of 100% EtOH to the remaining interphase and BCP phase.
- 16 Vortex samples vigorously and centrifuge at 12,000g for 5 minutes to pellet the protein.
- 17 

Remove supernatant and dry the pellet at room temperature for 1-2 minutes.

 - **Critical Step:** The protein pellet should not be dried excessively.
- 18 Add desired volume of SDS-Urea Buffer (at least 100 µl per 10 mg tissue), vortex, and allow protein pellet to dissolve at room temperature.
- 19 

To quantify protein concentrations, follow the manufacturer's protocol for the Bio-Rad RC/DC Protein Assay (5000121).

 - **Important point:** The RC component of the protein assay is necessary due to the presence of reducing agents in the SDS-urea buffer.
 - **Critical Step:** Protein samples in the SDS-urea buffer are diluted 10-fold to reduce the total starting amounts of protein and reducing agents in the assay. Protein standards are made using bovine serum albumin solubilized in the respective buffer and are subjected to the same dilution step as the unknown samples ensure consistency.