

Xiping Zhang (Esser lab) Protein Extraction RIPA Buffer Recipe:

Frozen muscle samples were homogenized in ratio of 1:10-20 (10-20ul/mg muscle) in ice-cold RIPA buffer (see list)

- 1% NP-40
- 0.5% sodium deoxycholate
- 0.1% SDS
- 50 mM sodium chloride
- 20 mM Tris-HCl (pH 7.6)
- 1 mM phenylmethylsulfonyl fluoride (PMSF)
- 5 mM benzamidine
- 1 mM EDTA
- 5 mM *N*-ethylmaleimide
- 50 mM sodium fluoride
- 25 mM  $\beta$ -glycerophosphate
- 1 mM sodium orthovanadate
- 10  $\mu$ l/ml protease inhibitor cocktail

Homogenates were then centrifuged at 17,860 *g* for 10 min at 4°C and the supernatant collected for analysis.