

Whole Cell Lysis Buffer:

12/20/03

Stock Solution	20ml	Final Concentration
1M KCl	1000ul	50mM
NP-40	200ul	1%
1M HEPES (pH7.8)	500ul	25mM
5mg/ml Leupeptin (SIGMA)	40ul	100ug/ml
10mg/ml Aprotinin (SIGMA)	40ul	20ug/ml
250 mM DTT	10ul	125uM
100 mM PMSF	200ul	1mM
40 mMNa ₃ VO ₄	500ul	1mM
dH ₂ O	17.5ml	

Total Protein Extraction from Tissues:

1. Add 1 ml WCL extraction buffer per 100 mg of tissue.
2. Homogenize tissues.
3. Transfer to 1.5ml eppendorf tubes.
4. Spin at 13,000 rpm for 10 minutes at 4°C.
5. Remove supernatant and save in another tube.
6. If necessary, centrifuge supernatant again.
7. Measure protein concentration with BCA method.

Or

1. Homogenize tissues in 1XPBS.
2. Spin at 3,000 rpm for 10 minutes at 4°C
3. Remove supernatant.
4. Add 1 ml WCL extraction buffer per 100 mg of tissues.
5. Sonication for 4X8 at output level 5.
6. Spin at 13,000 rpm for 10 minutes at 4°C.
7. Remove supernatant and save in another tube.
8. If necessary, centrifuge supernatant again.
9. Measure protein concentration with BCA method