Protocol

Extraction of Total Proteins from Plant Tissue (Small Scale)

Modified from:

*** It can be used for microrganisms or other tissues.*

Hurkman W. J. and Tanaka C. K. (1986). *Solubilization of Plant Membrane Proteins for Analysis by Two-Dimensional Gel Electrophoresis*. Plant Physiol. (81): 802-806

Use high pure grade salts such as Sigma, Merck..

1) Extraction Buffer:

Compounds	**Stock Solution**	**WC**	**15 mL**	**60 mL**
Tris HCl (1M, pH=7.5) (MW = 121.14)	60.6 g/0.5L	0.5 M	7.5 mL	30 mL
KCl (1M, MW= 74.56)	7.46 g/100mL	0.1 M	1.5 mL	6 mL
EDTA (0.5M, pH=8.0) (MW = 372.23)	18.6 g/100mL	0.05 M	1.5 mL	6 mL
Sucrose (MW = 342.3)		0.7 M	3.6 g	14.4 g
B-mercaptoethanol *		2% (v/v)	300 µL	
PMSF (MW = 174.2)*		2 mM	5.22 mg	0.021 g
PVPP *		1 %	0.15 g	
milliQ		qsq	15 mL	60 mL

**** Obs:***

i) The PMSF (2mM) should be previously dissolved in 300 uL of isopropanol, before been added to the extraction buffer.

ii) The PVPP is added as powder (1%), to the extraction buffer, just before using and should be homogenised.

iii) The β-mercaptoethanol (2%) is added to the extraction buffer, just before using and should be homogenised.

2) Solubilisation Buffer:

Compounds	**WC**	**10 mL**
Urea (MW = 60.06)	7.0 M	4.2 g
Tiourea (MW = 76.12)	2.0 M	1.52 g
DTT (MW = 154.3)	10 mM (= 0.01M)	15.4 mg
Triton X-100	0.01% (w/v)	1 mg
milliQ	qsq	10 mL

3) Wash Buffer (100% Methanol + 0.1M Ammonium acetate):

Compounds	**WC**	**100 mL**
Ammonium Acetate (MW = 77.08)	0.1M	0.78 g
Methanol (100%)	qsq	100 mL

Keep it at (-20ºC)

4) Precipitation Buffer (100% Acetona):

Compound	**100 mL**

Acetone (100%)	100 mL
Keep it at (-20ºC)	

Protocol:

i) Ground 100 mg of tissue using a mortar and pestle, under liquid nitrogen. Transfer the sample to safe lock centrifuge tubes (2 mL) ***Axygen (cat# MCT-500-C)*** and add 800 μL of extraction buffer + PVPP + β-mercaptoethanol). Homogenize (Vortex).

ii) Use an orbital shaker (150 rpm) to homogenize the samples for 30 min. at 4ºC. *Keep the tube in the horizontal position for vigorous shaking.*

iii) Add 1 volume (800 μL) of equilibrated phenol with 10mM de Tris-HCl (pH=8.0) ***(Sigma, cat# P4557).*** Homogenize the samples, using an orbital shaker (150 rpm), for 30 min. at 4ºC.

iv) Centrifuge (10.000 *g),* for 30 min. at 4ºC.

v) Recover the supernatant, transfer it to a new tube and add 1 volume (800 μL) of extraction buffer + PVPP + β-mercaptoethanol. Homogenize the samples, using an orbital shaker (150 rpm), for 30 min. at 4ºC.

vi) Centrifuge (10.000 *g),* for 30 min. at 4ºC.

vii) Repeat the steps *v* and *vi*. In this last step do not add PVPP and β-mercaptoethanol to the extraction buffer.

viii) Centrifuge (10.000 *g),* for 30 min. at 4ºC. Recover the supernatant.

ix) Add 1.6 mL of the wash buffer (100% methanol + 0.1 M Ammonium acetate) cold and keep the samples at -20ºC, overnight.

x) Centrifuge (16000 *g*) for 30 min. at 4ºC.

xi) Discard the supernatant and add 1.6 mL of wash buffer (100% methanol + 0.1M ammonium acetate) cold (-20ºC), without disturbing the pellet. Keep the tube in the freezer (-20ºC), for 1 hour (minimum).

xii) Centrifuge (16000 *g*) for 30 min. at 4ºC.

xiii) Repeat steps *xi* and *xii* (2 X).

xiv) Discard the supernatant and add 1.6 mL of precipitation buffer (100% acetone) cold (-20ºC), without disturbing the pellet. Keep the tube in the freezer (-20ºC), for 1 hour (minimum).

xv) Centrifuge (16000 *g*) for 30 min. at 4ºC.

xvi) Dry the pellet (Use a glass jar containing silica gel). Seal it very well and keep at 4ºC, overnight *(Do not let the sample dry for longer)*. Suspend it in 400 µL of solubilisation buffer, *(depending on the sample and yield).* Centrifuge (16.000 *g*), for 10 min., at 4ºC, to remove residual impurities. Transfer the supernatant to Eppendorf tubes.

xvii) *Desalt the samples using an Amicon®Ultra-0.5 filter device (**cat#UFC 5003BK**) from Merck 3000-10000 NMWL (depending on the cuttoff you are interested in using).*

xviii) Quantify the samples using the Bradford test.

xix) Prepare an SDS-PAGE gel to check the quantification of the protein obtained by the Bradford test. In the same gel, run also a BSA sample (2ug) for visual quantification. ***Obs:*** *It is common that the quantification by Bradford is not compatible with the gel, depending on the sample).* ***Obs: Make the correction of the protein concentration, based on the gel analysis. There are same subproducts resulting from the extraction that may absorb at 595nm.***

xx) Keep the samples at -80℃ till analysis.