Sample preparation

(1) Chemical

Lysis buffer (10ml)

Urea (9.5 M)	4.8 g
NP-40 (2%)	0.2 ml
Ampholine (2%) (pH 3.5 – 10)	0.2 ml
β-mercaptoethanol(5%)	0.5 ml
PVP-40	0.5 g
Water	9.10 ml

Note. This buffer may be stored frozen at –20°C. Do not continuously freeze and thaw it. Use an aliquot once and discard the remainder

(2) Method

a. Collect sample and homogenize in lysis buffer.

b. Centrifuge at 15,000 g for 10 min

c. Take the supernatant and centrifuge at 15,000 g for 10 min, again

d. Take the supernatant and use for 2D-PAGE