

中國醫藥大學蛋白質體核心實驗室實驗技術

2D Chemical Solution Preparation – For GE System

* 1 D solution

1. 1% Bromophenol blue stock solution-- 10 ml

	Final concentration	Amount
Bromophenol blue	1 %	100 mg
Tris-base	50 mM	60 mg
ddH ₂ O	-	add to 10 ml

2. Urea rehydration stock solution – 25 ml

	Final concentration	Amount
Urea(FW 60.06)	8M	12 g
CHAPS	2 %	0.5 g
1% Bromophenol blue	0.002%	50 µl
IPG buffer	0.5%	125 µl
ddH ₂ O	-	to 25 ml

分裝後置於-20°C。

使用前再加 DTT, 7 mg/2.5 ml rehydration stock solution。

3. Mineral Oil

4. Sample volume applied (µl)

Sample volumes for different Immobiline DryStrip gel lengths.

Immobiline DryStrip gel length (cm)	Sample volume applied (µl)
7	125
11	200
13	250
18	340
24	450

例：13 cm Dry strip Sample volume=Protein sample+ rehydration solution= 250 µl



中國醫藥大學蛋白質體核心實驗室實驗技術

* 2 D equilibration solution

1. 1.5 M Tris-HCl pH8.8 – 500 ml

	Final concentration	Amount
Tris-base(FW 121.4)	1.5 M	90.85 g
ddH ₂ O	-	375 ml
HCl	-	adjust to pH8.8
ddH ₂ O	-	125 ml

Filter solution through a 0.45-μM filter. Store at 4°C.

2.10% SDS solution – 50 ml

	Final concentration	Amount
SDS(FW 288.38)	10%	5 g
ddH ₂ O	-	to 50 ml

Filter solution through a 0.45-μM filter. Store at room temperature.

3. SDS equilibration buffer solution—200 ml

	Final concentration	Amount
Urea(FW 60.06)	6M	72.1g
1.5 M Tris-HCl pH8.8	75 mM	10 ml
Glycerol(87% w/w)	29.3(v/v)	69.2 ml
SDS(FW 288.38)	2%	4g
1% Bromophenol blue	0.002%	400 μl
ddH ₂ O	-	to 200 ml

分裝後置於-20°C。

* 2D Gel running solution

1. 10% ammonium persulfate —1 ml

	Final concentration	Amount
ammonium persulfate	10%	0.1 g
ddH ₂ O	-	to 1 ml

新鮮配製。

2. 10X SDS running buffer—3L

	Final concentration	Amount
Tris-base(FW 121.4)	25 mM	90.9g
Glycine (FW 75.07)	192 mM	432.3g
SDS(FW 288.38)	0.1%	30g
ddH ₂ O	-	to 3 L

