Miscellaneous Solutions

1x WB 1x Ni-NTA Chromatography Loading/Wash Buffer, pH 8.0

Chemical	FW	to add for 1 l
300 mM NaCl	58.44	17.5 g
20 mM HEPES	238.3	4.77 g
20 mM Imidazole	68.08	1.36 g

Bring to 950 ml with H_20, bring to pH 8.0 ($\sim$35 drops 10 N NaOH), then bring to 1 l.

10x WB 10x Ni-NTA Chromatography Loading/Wash Buffer, pH 8.0

Chemical	FW	to add for 1 l
3 M NaCl	58.44	175.3 g
200 mM HEPES	238.3	47.7 g
200 mM Imidazole	68.08	13.6 g

Bring to 950 ml with H_20, bring to pH 8.0 ($\sim$100 drops 10 N NaOH), then bring to 1 l.

1x EB Ni-NTA Chromatography Elution Buffer, pH 8.0

Chemical	FW	to add for 1 l
300 mM NaCl	58.44	17.5 g
20 mM HEPES	238.3	4.77 g
250 mM Imidazole	68.08	17.0 g

Bring to 975 ml with H_20, bring to pH 8.0 ($\sim$50 drops 37% HCl), then bring to 1 l.

Crystallization Buffer 10 mM NaCl, 10 mM HEPES, pH 7.4

Chemical	FW	to add for 1 l
10 mM NaCl	58.44	0.58 g
10 mM HEPES	238.3	2.38 g

Bring to 975 ml with H_20, bring to pH 7.4 ($\sim$7 drops 10 N NaOH), then bring to 1 l.

NMR Buffer 50 mM NaCl, 10 mM Phosphate pH 7.4, 0.02% NaN_3

Chemical	FW	to add for 1 l
50 mM NaCl	58.44	2.92 g
2.3 mM Monosodium phosphate, monohydrate	137.99	0.31 g
7.7 mM Disodium phosphate, heptahydrate	268.07	2.07 g
0.02% NaN_3 (Sodium Azide)	65.01	0.2 g

Bring to 1 l.

Coomassie Destain #1 Rinse Solution

Chemical	to add for 1 l
95% Ethanol	280 ml
Glacial Acetic Acid	100 ml
Water	620 ml

Coomassie Destain #2 Drying Solution

Chemical	to add for 1 l
Glycerol	100 ml
Glacial Acetic Acid	50 ml
Water	850 ml

Anion Exchange Buffer A 10 mM NaCl, 30 mM Tris, pH 8.0

		to add for 1 l	
Chemical	FW	4°C	25°C
10 mM NaCl	58.44	0.58 g	0.58 g
30 mM Tris			
Tris-HCl	157.6	3.97 g	2.67 g
Tris (Base)	121.1	0.58 g	1.59 g

Anion Exchange Buffer B 1 M NaCl, 30 mM Tris, pH 8.0

		to add for 1 l	
Chemical	FW	4°C	25°C
1 M NaCl	58.44	58.4 g	58.4 g
30 mM Tris			
Tris-HCl	157.6	3.97 g	2.67 g
Tris (Base)	121.1	0.58 g	1.59 g

1M Tris, pH 8 Will be pH 8 when diluted to 30 mM at the given temperature

		to add for 1 l	
Chemical	FW	4°C	25°C
1 M Tris			
Tris-HCl	157.6	132.2 g	88.8 g
Tris (Base)	121.1	19.4 g	53.0 g

5x M9 Salts - Based on Maniatis, A2.2

Chemical	FW	to add for 1 l
$Na_2HPO_4 \cdot 7H_2O$	268.07	64 g
KH_2PO_4	136.09	15 g
NaCl	58.44	2.5 g
NH_4Cl (leave out for isotopic labeling)	53.50	5.0 g

Sterilize by autoclaving.

1 M $MgSO_4$ - Based on Maniatis, A2.2

Chemical	FW	to add for 100 ml
$MgSO_4$	120.37	12.0 g

Sterilize by autoclaving.

1 M $CaCl_2$ - Based on Maniatis, A2.2

Chemical	FW	to add for 100 ml
$CaCl_2 \cdot H_2O$	147.02	14.7 g

Sterilize by autoclaving.

M9 Minimal Media - Based on Maniatis, A2.2

Ingredient	to add for 1 l
H_2O (Sterile)	775 ml
5x M9 Salts (Sterile)	200 ml
1 M $MgSO_4$ (Sterile)	2 ml
20% Glucose Solution (Filter Sterilized)	20 ml
1 M $CaCl_2$ (Sterile)	0.1 ml
1000x Antibiotic (Filter Sterilized)	1 ml
if cells are Δleu	
20 mg/ml L-Leucine (Filter Sterilized)	2 ml

To make M9 agar plates, make agar at 20 g/l and autoclave. Then make media as above, but use the 20 g/l agar instead of H_2O to obtain a final agar concentration of 15 g/l.

1000X DNAse/RNAse stock :

Ribonuclease A (Bovine Pancreas, Sigma R-5500)	10 mg
Deoxyribonuclease I (Bovine Pancrease, Sigma D-4527, 2000 units/mg)	5 mg
PBS	1 ml

0.2 μm filter sterilize.