

Protein expression and purification reagents

Buffer A (20 mM Tris-HCl pH 8.0, 350 mM NaCl, 20 mM imidazole)

-20 ml 1M Tris-HCl, pH 8.0

-70 ml 5M NaCl

-1.36 g imidazole

-dH₂O to 1L

Filter sterilize.

Buffer B (20 mM Tris-HCl pH 8.0, 350 mM NaCl, 400 mM imidazole)

-20 ml 1M Tris-HCl, pH 8.0

-70 ml 5M NaCl

-27.2 g imidazole

-dH₂O to 1L

Filter sterilize.

Buffer C (20 mM Tris-HCl pH 8.0, 50 mM NaCl)

-20 ml 1M Tris-HCl, pH 8.0

-10 ml 5M NaCl

-dH₂O to 1L

Filter sterilize.

Buffer D (20 mM Tris-HCl pH 8.0, 100 mM NaCl)

-20 ml 1M Tris-HCl, pH 8.0

-20 ml 5M NaCl

-dH₂O to 1L

Filter sterilize.

Buffer E (20 mM Tris-HCl pH 8.0, 1 M NaCl)

-20 ml 1M Tris-HCl, pH 8.0

-200 ml 5M NaCl

-dH₂O to 1L

Filter sterilize.

Buffer F (20 mM Tris-HCl pH 8.0, 350 mM NaCl)

-20 ml 1M Tris-HCl, pH 8.0

-70 ml 5M NaCl

-dH₂O to 1L

Filter sterilize.

Sumoylation storage buffer (20 mM Tris pH 7.5, 50 mM NaCl, 10% glycerol)

20 ml 1M Tris-HCl pH 7.5

50 ml 5M NaCl

100 ml glycerol
dH2O to 1L
Filter sterilize.

TGI buffer (20 mM Tris-HCl pH 7.5, 250 mM NaCl, 10% glycerol, 20 mM imidazole)

Per litre:

20 ml 1M Tris-HCl pH 7.5

50 5M NaCl

100 ml glycerol

1.36 g imidazole

900 ml dH2O

Mix ingredients using a stir bar. Bring to 1L with dH2O. Filter sterilize into 1L bottle.

TGI-500 (20 mM Tris-HCl pH 7.5, 250 mM NaCl, 10% glycerol, 500 mM imidazole)

Per litre:

20 ml 1M Tris-HCl pH 7.5

50 5M NaCl

100 ml glycerol

34 g imidazole

900 ml dH2O

Mix ingredients using a stir bar. Bring to 1L with dH2O. Filter sterilize into 1L bottle.

Buffer G (20 mM HEPES pH 7.4)

40 ml of 1 M HEPES-KOH pH 7.4

dH2O to 2L filter sterilize.

Buffer H (20 mM HEPES pH 7.4, 1M NaCl)

20 ml of 1 M HEPES-KOH pH 7.4

200 ml of 5M NaCl

dH2O to 1 L filter sterilize.

Buffer J (20 mM sodium citrate, 50 mM NaCl)

-10 ml of 1M Na Citrate

-5ml of 5M NaCl

dH2O to 500 ml, filter sterilize

Buffer K (20 mM sodium citrate, 1M NaCl)

-10 ml of 1M Na Citrate

-100 ml of 5M NaCl

dH2O to 500 ml, filter sterilize

Buffer L (20 mM HEPES pH 7.5, 1 mM EDTA, 2 mM CHAPS)

-40 ml HEPES pH 7.5

-4 ml 500 mM EDTA

-2.458 g CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate)
dH2O to 2L

Buffer M (20 mM HEPES pH 7.5, 1 mM EDTA, 2 mM CHAPS, 1M NaCl)

-10 ml HEPES pH 7.5
-1 ml 500 mM EDTA
-0.615 g CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate)
-100 ml of 5M NaCl
-dH2O to 500 ml

Buffer C+GLYC (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 10% glycerol)

-20 ml 1M Tris-HCl, pH 8.0
-30 ml 5M NaCl
-100 ml glycerol
-dH2O to 1L
Filter sterilize

Buffer L*GLYC (20 mM HEPES pH 7.5, 1 mM EDTA, 2 mM CHAPS, 50 mM salt 10% glycerol)

-20 ml HEPES pH 7.5
-2 ml 500 mM EDTA
-1.229 g CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate)
-100 ml glycerol
-10 ml 5M NaCl
dH2O to 1L
Filter sterilize

4M KCl

-dissolve 298.2 g KCl in 900 ml dH2O (might need heating to get fully in solution)
-bring volume up to 1L with dH2O
-sterilize by autoclaving

1x Pfu Storage buffer (50 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT, 10% glycerol, 0.1% NP40) (ran over column)

50 ml of 1M Tris-HCl pH8.0
200 µl of 0.5M EDTA
1 ml 1M DTT
100 ml glycerol
10 ml of 10% NP-40

dH2O to 1L, filter sterilize.

1x Pfu Storage buffer (50 mM Tris-HCl pH 8.0, 0.1 mM EDTA, 1 mM DTT, 50% glycerol, 0.1% NP40) (for concentrating and freezing)

50 ml of 1M Tris-HCl pH8.0

200 μ l of 0.5M EDTA

1 ml 1M DTT

500 ml glycerol

10 ml of 10% NP-40

dH2O to 1L, filter sterilize.

DBD column buffer (20 mM Tris-HCl pH 7.5, 400 mM NaCl, 50 μ M ZnCl₂, 20 mM imidazole)

-20 ml 1M Tris-HCl, pH 7.5

-80 ml 5M NaCl

-50 μ l 1M ZnCl₂

-1.36 g imidazole

-dH2O to 1L

Filter sterilize.

For elution buffer, dissolve 0.27 g per 10 ml. Filter sterilize