

REFERENCES



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APPENDIX

APPENDIX PREPARATION OF REAGENTS

10% Ammonium persulfate (w/v) [5 mL]

Ammonium persulfate 0.5 g

Add ddH₂O to make 5 mL and store at 4 °C.

Stable for months in a capped tube in 4 °C refrigerator.

Cell culture freezing medium [10 mL]

Inactivated fetal bovine serum 9 mL [90%]

DMEM/F12 medium 500 µL [5%]

Sterilized DMSO 500 µL [5%]

(Add DMSO immediately before use.)

Coomassie De-staining solution [1 L]

Glacial acetic acid 100 mL

Methanol 450 mL

ddH₂O 450 mL

DMEM/F12 medium (GIBCO invitrogen)

With L-glutamine and pyridoxine hydrochloride.

Without HEPES buffer and sodium bicarbonate.

Measure out 5% less distilled water than desired total volume of medium, using a mixing container that is as close to the final volume as possible.

Add powdered medium to 15 to 30 °C (room temperature) water with gentle stirring. (Do not heat water.)

Rinse out inside of the package to remove all traces of powder.

Add 2.43 g of NaHCO₃ per liter of medium.

Dilute to a desired volume with distilled water. Stir until dissolve. (Do not over-mix.)

Adjust pH of medium to 0.1-0.3 below desired final working pH*: use of 1 N NaOH or 1 N HCl is recommended. (Add slowly with stirring.) After pH has been adjusted, keep container closed until medium is filtered.

Sterilize immediately by membrane filtration. (Positive pressure recommended.)

*pH units will usually rise 0.1-0.3 upon filtration.

6x DNA loading buffer [10 ML]

Bromophenol blue	25 mg [0.25%]
Xylene cyanol FF	25 mg [0.25%]
Glycerol	3.3 mL [30%]
ddH ₂ O	6.7 mL

Low-serum RPMI 1640 medium [1 L]

RPMI 1640 medium (serum-free)	950 mL
Inactivated horse serum	30 mL [3%]
Inactivated fetal bovine serum	20 mL [2%]
100X Penicillin and Streptomycin	10 mL [1X]

Low-serum RPMI 1640 medium with 20 μ M Retinoic acid [1 L]

RPMI 1640 medium (serum-free)	950 mL
Inactivated horse serum	30 mL [3%]
Inactivated fetal bovine serum	20 mL [2%]
100 μ M Retinoic acid	200 μ L [1X]
100X Penicillin and Streptomycin	10 mL

Normal-serum RPMI 1640 [1 L]

RPMI 1640 medium (serum-free)	850 mL
Inactivated horse serum	100 mL [10%]
Inactivated fetal bovine serum	50 mL [5%]
100X Penicillin and Streptomycin	10 mL [1X]

1X Phosphate buffer saline (1X D-PBSA)

Dulbecco's Phosphate Buffered Saline, without Ca ²⁺ and Mg ²⁺	
NaCl [MW = 58.44]	8 g [136.9 Mm]

KCl [MW = 74.55]	0.2 g [2.68 mM]
KH ₂ PO ₄ [MW= 136.1]	0.2 g [1.47 mM]
Na ₂ HPO ₄ [MW = 142]	1.14 g [8.06]

Dissolve in 1 litre distilled water and adjust to pH 7.4. This solution was then autoclaved before use.

10 mg/ mL Poly-D-lysine hydrobromide

Poly-D-lysine hydrobromide [MW □ 300,000]	5 mg
Sterilized deionized H ₂ O	50 mL

Store at -20 °C until used.

5x Protein sample buffer

1M Tris pH 6.8	0.6 mL [60 mM]
100% Glycerol	2.5 mL [25%]
10% SDS	2.0 mL [2%]
β-mercaptoethanol [MW= 78.13]	0.5 mL
1% Bromophenol blue	1.0 mL [0.1%]
ddH ₂ O	3.4 mL

Stable for week in the 4oC refrigerator or for months at -20°C.

RIPA buffer [100 mL]

1 M Tris-HCl pH 7.6	2.5 mL [25 mM]
NaCl [MW = 58.44]	0.88 g [150 mM]
NP-40	1.0 mL [1%]
Sodium deoxycholate [MW = 414.56]	1.0 g [1%]
10% SDS	1.0 mL [0.1%]

Add ddH₂O to make 100 mL and store at 4 °C.

RPMI 1640 medium (Serum-free) (GIBCO invitrogen)

With L-glutamine and pyridoxine hydrochloride.

Without HEPES buffer and sodium bicarbonate.

Measure out 5% less distilled water than desired total volume of medium, using a mixing container that is as close to the final volume as possible.

Add powdered medium to 15 to 30 °C (room temperature) water with gentle stirring. (Do not heat water.)

Rinse out inside of the package to remove all traces of powder.

Add 2.0 g of NaHCO₃ per liter of medium.

Dilute to a desired volume with distilled water. Stir until dissolve. (Do not over-mix.)

Adjust pH of medium to 0.1-0.3 below desired final working pH*: use of 1 N NaOH or 1 N HCl is recommended. (Add slowly with stirring.) After pH has been adjusted, keep container closed until medium is filtered.

Sterilize immediately by membrane filtration. (Positive pressure recommended.)

*pH units will usually rise 0.1-0.3 upon filtration.

10x Running electrophoresis buffer [1L]

Tris-Base [MW = 121.14] 30.2 g [250 mM]

Glycine [MW = 75.07] 144 g [1.92 M]

SDS [MW = 288.38] 10 g [10%]

H₂O to make 1 litre

pH should be approximately 8.3. Stable indefinitely at room temperature.

10% SDS (w/v) [100 mL]

SDS 10 g

Add ddH₂O to make 100 mL.

Store at room temperature.

Stock 5 mM camptothecin

Camptothecin [MW = 348.36]	100 mg
Dissolve in sterilize DMSO	10 mL
Add sterilize deionized H ₂ O to make	57.4 mL
Store the stock solution at -20 °C.	

stock 10 mg/ mL MTT

ultra pure MTT [MW= 414.33]	50 mg
sterilized D-PBSA	5 mL
Store in 4 °C refrigerator.	

100 mM Stock retinoic acid

all-trans-retinoic acid [MW = 300.44]	50 mg
sterilize DMSO	1.6642 mL
Store in capped tube at -20 °C.	

Stripping buffer (for reprobing western blots) [200 mL]

10% SDS	40 mL [0.02%]
0.5 M Tris-HCl pH 6.8	25 mL [62.5 Mm]
Ultra pure water	135 mL
β-mercaptoethanol (under fumehood)	1.6 mL

Tris acetate EDTA buffer (TAE buffer) [1 L]

Tris-Base [MW = 121.14]	48.4 g [0.4 M]
Glacial acetic acid	11.4 mL
EDTA, disodium salt [MW = 372.24]	3.7 g [0.01 M]
Adjust pH to 8.5 + 0.2 and add deionized H ₂ O to make 1 litre	

10X Tris buffered saline (10X TBS)

Tris-Base [MW = 121.14]	12.114 g	[100 mM]
NaCl [MW = 58.44]	87.66 g	[1.5 M]
Adjust pH to 7.5 and add H ₂ O to make 1 litre		

Tris buffered saline tween (TBST)

10X TBS	100 mL	[1X]
Tween 20	500 μ L	[0.05%]
ddH ₂ O	900 mL	

1 M Tris-HCl, pH 6.8 [100 mL]

Tris-Base [MW = 121.14] 12.1 g

Add to 50 mL ddH₂O.

Add concentrated HCl slowly to pH 6.8 (about 8 mL).

Add ddH₂O to make 100 mL.

1.5 M Tris-HCl, pH 8.8 [100 mL]

Tris-Base [MW = 121.14] 18.171 g

Add to 50 mL ddH₂O.

Add concentrated HCl slowly to pH 8.8 (about 4 mL).

Add ddH₂O to make 100 mL.

Westernblot transferring buffer [1L]

1X Running electrophoresis buffer 800 mL

Methanol 200 mL

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