Appendix B

Appendix B

Composition and Preparation of Solution

1. Composition of RPMI Medium 1640

RPMI medium 1640 (1X) liquid are contains 25 mM HEPES buffer and L-glutamine and other components.

Components	**MW**	**Concentration (mg/L)**	**mM**
Amino acid			
glycine	75	10	0.133333
L-Arginine	174	200	1.149425
L-Asparagine	132	50	0.378788
L-Aspartic acid	133	20	0.150376
L-Cystine 2HCl	313	65	0.207668
L-Glutamic Acid	147	20	0.136054
L-Glutamine	146	300	2.054794
L-Histidine	155	15	0.096774
L-Hydroxyproline	131	20	0.152672
L-Isoleucine	131	50	0.381679
L-Lysine hydrochloride	146	40	0.273973
L-Methionine	149	15	0.100671
L-Phenylalanine	165	15	0.090909
L-Proline	115	20	0.173913
L-Serine	105	30	0.285714
L-Threonine	119	20	0.168067
L-Tryptophan	204	5	0.02451
L-Tyrosine disodium salt dihydrate	261	29	0.111111
L-Valine	117	20	0.17094

Components (continue)	**Molecular weight**	**Concentration (mg/L)**	**mM**
Vitamins			
Biotin	244	0.2	0.00082
Choline chloride	140	3	0.021429
D-Calcium pantothenate	477	0.25	0.000524
Folic Acid	441	1	0.002268
Niacinamide	122	1	0.008197
Para-Aminobenzoic Acid	137	1	0.007299
Pyridoxine hydrochloride	206	1	0.004854
Riboflavin	376	0.2	0.000532
Thiamine hydrochloride	337	1	0.002967
Vitamin B12	1355	0.005	0.000004
i-inositol	180	35	0.194444
Inorganic Salts			
Calcium nitrate ($Ca(NO_3)_2$ $4H_2O$)	236	100	0.423729
Magnesium Sulfate ($MgSO_4$) (anhydrous.)	120	48.84	0.407
Potassium Chloride (KCl)	75	400	5.333334
Sodium Bicarbonate ($NaHCO_3$)	84	2000	23.809525
Sodium Chloride (NaCl)	58	5300	91.37931
Sodium Phosphate dibasic (Na_2HPO_4) anhydrous	142	800	5.633803
Other components			
D-Glucose (Dextrose)	180	2000	11.111111
Glutathione (reduced)	307	1	0.003257
HEPES	238	5958	25.033613
Phenol Red	376.4	5	0.013284

2. Preparation of salt solution for isolation LDL

Reagents

1. Ethylenediaminetetraacetic acid (EDTA)
2. Sodium chloride (NaCl)
3. Potassium bromide (Kbr)
4. Distilled water (DW)

Preparation

1. Preparation of stock salt solution (SSS) (D = 1.346 g/ml)
   - NaCl 153 g/l
   - KBr 354 g/l
   - EDTA 0.1 mg/ml
2. Preparation of dilution salt solution (DSS) (D = 1.005 g/ml)
   - NaCl 8.775 g/l
3. Preparation of gradient salt solution

   D = 1.063 g/ml

   - 4 ml of SSS + 19.5 ml of DSS

   D = 1.019 g/ml

   - 1 ml of SSS + 23.4 ml of DSS

   D =1.006 g/ml)

   - 0.5 ml of SSS + 170 ml of DSS

3. Preparation of 0.1 M phosphate buffer saline (PBS), pH 7.3 –7.4

- Sodium phosphate dibasic (Na_2HPO_4) 1.292 g/l
- Sodium phosphate monobasic (H_2NaO_4) 0.235 g/l
- Sodium chloride (NaCl) 8.7 g/l

All chemical were mixed with DW and adjusted pH 7.3-7.4 by sodium hydroxide (NaOH)

4. Solution for measurement of LDL protein

- 2 mg of bovine serum albumin (BSA) were mixed in DW 1 ml.
- Protein reagent solution was prepared by mix of 4% w/v of copper (II) sulfate (Cu_2SO_4) and bicinchoninic acid solution at ratio1:50.

5. 0.89 M EDTA Na_2 : 2.99 mg of EDTA in 1 ml of NaOH solution

6. 160.2 nM Cu_2SO_4

0.0255 g of Cu_2SO_4 in 1 ml of DW

7. solutions for 1% agarose gel electrophoresis of LDL

- 0.05 M Barbital buffer

  dissolved of 1.84 g diethyl barbituric acic and 10.27 g sodium diethyl barbiturate and make up to 1 litre with DW

- Sample buffer (10 ml)

  mixed of 10% (v/v) glycerol and 0.05% (w/v) bromophenol blue and make up to 10 ml of barbital buffer

- Fixation solution (500 ml)

  mixed of 25 % (v/v) Isopropanol and 10% (v/v) glacial acetic acid and make up to 500 ml of DW

- Staining solution (1 litre)

  mixed of 10% (v/v) glacial acetic acid and 0.006% (w/v) commassie blue and make up to 1 litre of DW

- Destaining with 10% acetic acid

Chemical lists

Acrylamide PAGE (MW=71.08)	Amersham, Biosciences
Agarose (Biotecnology grade)	AMRESO
Albumin, bovine serum (BSA)	Sigma, USA
Ammoniumperoxodisulfate (MW=228.20)	Sigma-Aldrich, USA
Amphotericin B (MW=924.1)	Sigma, USA
Ascorbic acid, r-irradiated	Sigma, USA
Bicinchoninic acid	Sigma, USA
Bromophenol Blue (Sodium salt) Ultrapure MW = 691.95	USB
Buffer solution pH 4, 7,10	CARLO ERBA
Chloroform	Sigma, USA
DAB substrate kit	ZyMED, Invitrogen
Deferoxamine mesylat (MW = 656.8)	Sigma, USA
Diethypyrocarbonate (DEPC) (MW = 162.14)	AMRESO
Dimethyl sulfoxide (MW = 78.13)	Riedel-de Haen
EDTA-Na_2.$2H_2O$, Ultrapure (MW = 372.4)	USB
Fetal Bovine Serum	GIBCO ®Invitrogen
Formaldehyde, 37 wt% (MW = 30.03)	ALDRICH, USA
Glycerol, Ultrapure (MW = 92.09)	USB
Goat F (ab)2 Anti-Rabbit Ig's, HRP Conjugate	BIOSOURCE
Mercaptoethanol (MW = 78.13)	Amersham Bioscience
Methylene bisacrylamide (MW = 154.17)	Amersham Bioscience
N,N,N',N'-Tetramethyl ethylene-diamine (MW=116.21)	Amersham Bioscience
Normal Goat Serum (10 ml)	ZYMED, Invitrogen
Phenylmethyl Sulfonyl Fluoride, Ultrapure	USB
Potassium chloride (KCl) (MW = 74.55)	BDH, England
Potassium bromide (KBr) (MW = 119)	BDH, England
Propanolol	USB

Chemical lists (continue)

Rabbit anti-phospho-p38 MAPK (Thr 180/Tyr 182)	Invitrogen, Carlsbae
RMPI medium 1640	GIBCO ®
Sodium azide (MW = 65.01)	BDH, England
Sodium borohyride (MW = 37.83)	Sigma-Aldrich,USA
Sodium chloride (NaCl) (MW = 58.44 g/mol)	BDH, England
Sodium Dodecyl sulfate, Ultrapure (MW = 288.38)	USB
Sodium phosphat monobasic, anhydrous (MW = 119.98)	Sigma, USA
Trichloroacetic acid	Sigma, USA
Tris (Tris [hydroxymethy]amino methane) ,Ultrapure (MW = 121.14)	USB
1,1,3,3-Tetramethyoxypropane, 99% (MW = 164.2)	Sigma-Aldrich,USA
2-Thiobarbituric acid minimum 98% (MW = 144.15)	Sigma-Aldrich,USA