

Appendix

1. Reagent for ELISA

Coating buffer, pH 9.6

Na ₂ CO ₃	0.975	g
NaHCO ₃	1.465	g
Deionized water	500	ml

PBS, 10X

NaCl	80	g
KH ₂ PO ₂	2	g
Na ₂ HPO ₄	11.5	g
KCl	2	g
Deionized water	1	L

PBS-T

PBS, 10X	100	ml
Deionized water	900	ml
Tween 20	0.5	ml
Neutral red	2.0	ml

0.1 M Citric acid

Citric acid, monohydrate	2.1	g
Deionized water	100	ml

0.2 M Na₂HPO₄

Na ₂ HPO ₄	2.84	g
Deionized water	100	ml

Stock OPD

o-phnylenediamine hydrochloride	50	mg
Absolute methanol	5	ml

Substrate solution

	1 plate	2 plates	3 plates	
0.1 M Citric acid	6.07	12.15	18.22	ml
0.2 M Na ₂ HPO ₄	6.43	12.85	19.28	ml
Mix and discard	0.5	1.0	1.5	ml
H ₂ O ₂ , 30%	5	10	15	μl
Stock OPD	0.5	1.0	1.5	μl

8 N Sulfuric acid

H ₂ SO ₄ , conc.	22.4	ml
Deionized water to make	100	ml

Blocking buffer (20 ml)

BSA	1	g
PBS	20	ml

Washing buffer (1L)

Tween 20	500	μ l
PBS	1	L

2. Reagents for SDS-PAGE and Western Blot analysis**Acrylamide stock (30% Acrylamide/0.8% Bis)**

Acrylamide (Promega)	30	g
N', N'-Bis-methylene-acrylamide (Sigma)	0.8	g
Deionized water to make	100	ml
Store at 4°C in the dark		

Buffer for separating gel (1.5 M Tris/0.4% SDS, pH 8.8)

Tris base (Sigma)	90.85	g
Deionized water	250	ml
10% SDS	20	ml
Adjust to pH 8.8 with conc. KCl		
Deionized water to make	500	ml

Buffer for stacking gel (1 M Tris, pH 6.8)

Tris base	12.1	g
Deionized water	50	ml
Adjust to pH 6.8 with conc. HCl		
Deionized water to make	100	ml

10% Ammonium persulfate (APS, stored at 4°C)

Ammonium persulfate	0.1	g
Deionized water	1.0	ml

10% SDS

SDS	10.0	g
Deionized water	100	ml

Sample buffer (2X)

1 M Tris (pH 6.8)	6.25	ml
Glycerol	10	ml
SDS	5	g
1% bromphenol blue	1	ml
Deionized water to make	50	ml

10x Running buffer

Tris base	30.3	g
Glycine	144	g
Deionized water to make	1	L

Transfer buffer (25 mM Tris, 192 mM Glycine, 20% Methanol)

10x Running buffer	100	ml
Absolute methanol	200	ml
Deionized water to make	1	L

DAB substrate

3,3'-diaminobenzidine tetrahydrochloride	6	mg
50 mM Tris, pH 7.6	10	ml
30% H ₂ O ₂	10	μl

50 mM Tris pH, 7.6

1 M Tris	5	ml
Deionized water	50	ml
Adjust to pH 7.6 with 50% HCl		
Deionized water to make	100	ml

Staining solution

Coomassie blue	2.5	g
Glacial Acetic acid	100	ml
Absolute methanol	100	ml
Deionized water to make	1	L

Destaining solution

Glacial acetic acid	100	ml
Absolute methanol	100	ml
Deionized water to make	1	L

Blocking solution (5% non fat milked with 0.1% Tween 20 in PBS)

10X PBS	10	ml
Deionized water	90	ml
Tween 20	0.1	ml
Non fat milked	5	g

Washing solution (0.05% Tween in PBS)

10X PBS	200	ml
Deionized water	1800	ml
Tween 20	1	ml

Running buffer

1X Running buffer	100	ml
Deionized water	890	ml
10% SDS	10	ml

Separating gel for gradient gel (0.75 mm thick gel, 15 ml)

Solution	8%	20%
1.5 M Tris/0.4%SDS (ml)	3.75	3.75
30% Acrylamide/0.8%Bis (ml)	4	10
Deionized water (ml)	7.2	1.2
10% APS (μ l)	75	75
TEMED	7.5	7.5

Stacking gel, 4% (5 ml)

1 M Tris, pH 6.8	0.625	ml
30% acrylamide/0.8% Bis	0.7	ml
Deionized water	3.5	ml
10% SDS	50	μ l
10% APS	50	μ l
TEMED	5	μ l

Elution buffer (1 L)

Tris base	3.0	g
Glycine	14.4	g
SDS	1.0	g
Deionized water	1	L

3. Reagent and media for hybridoma production**Incomplete Iscove's medium, 1000 ml**

Iscove's powder medium	17.7	g
NaHCO ₃	3.024	g
Deionized water to make	1	L

0.2% Trypan blue

Trypan blue	0.02	g
Deionized water	10	ml

4.25% NaCl, (5X saline)

NaCl	2.125	g
Deionized water	50	ml

Trypan blue (working)

0.2% Trypan blue	4	parts
5X saline	1	part

Red cell lysis buffer

NH ₄ Cl	4.145	g
KHCO ₃	0.5	g
EDTA	0.0186	g

Dissolve with 450 ml distilled water and adjust to pH 7.2. Then add distilled water to 500 ml, filter and store at 4°C

0.6% 2-mercaptoethanol (2-ME)

Iscove's medium	5	ml
2-mercaptoethanol	30	μl

Filter with 0.2 μm Millipore, and aliquot 1 ml/cryotube, freeze at -20°C

Complete medium, 200 ml

Iscove's medium	180	ml
Fetal calf serum	20	ml
Gentamicin (stock 40 mg/ml)	200	μl
Fungizone (stock 2.5 mg/ml)	200	μl

Complete media with BM condiMed H1 (200 ml)

Iscove's medium	160	ml
Fetal calf serum	20	ml
BM condiMed H1	20	ml
Gentamicin (stock 40 mg/ml)	200	μl
Fungizone (stock 2.5 mg/ml)	200	μl

Hybridoma selective medium, HAT or HT (200 ml)

Medium NCTC-109	20	ml
Incomplete iscove's medium	156	ml
Fetal calf serum	20	ml
Gentamicin (40mg/ml)	200	μl
Fungizone (2.5 mg/ml)	200	μl
0.6% 2-ME	120	μl
HAT or HT (100X)	2	ml

HAT medium with BM condiMed H1 (100 ml)

Incomplete iscove's medium	80	ml
Fetal calf serum	10	ml
BM condiMed H1	10	ml
Gentamicin (40mg/ml)	100	μl
Fungizone (2.5 mg/ml)	100	μl

Freezing medium

Fetal calf serum	4	ml
DMSO	2	ml
Iscove's medium	14	ml

4. Reagents for Beaver's Standard direct smear

2 N Na₂SO₄

Na ₂ SO ₄ · 10 H ₂ O	32.22	g
Glycerol	50	ml

Deionized water to make	100	ml
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1 N BaCl₂

BaCl ₂	10.41	g
Glycerol	50	ml
Deionized water to make	100	ml

5. Reagents for agar plate culture method

*Nutrient agar (Difco, USA)	2.3	g
Deionized water	100	ml

Mixed and boiled at 100°C for 2 minutes, poured in double walled Petri dish
10-12 ml/plate

***Formular per liter of nutrient agar**

Bacto beef extract	3	g
Bactopeptone	5	g
Bacto agar	15	g

Vita

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