

Reaction Mixtures, Media and Solutions:

These are used by me in my work;

5' End labeling:

Use T4 Polynucleotide kinase.

10 x buffers:

0.5M Tris-Cl pH 8,

0.1 M MgCl₂,

0.1M DTT,

3' Recessive end labeling:

One can use Klenow fragment, T4 DNA-pol or T7 DNA-pol,

Use 10x Klenow Buffer: containing 10x Hepes buffer pH 6.6.

0.5 M Tris-Cl pH 7.6,

0.1 M Mg Cl₂,

RNA labeling:

10x Transcription buffer:

0.4 M Tris-Cl pH 8,

0.08 M Mg Cl₂,

0.5 M NaCl,

0.02 M Spermidine-HCl.

5' end labeling:

10x buffer:

0.5 Tris-Cl pH 8,

0.1 M Mgcl₂,

0.1 M DTT (Dithiothretol).

Use T4 polynucleotide kinase.

Labeling 3' overhangs:

10x buffer:

1M Potassium cocodylate pH 7.2,

20mM cacl₂,

2mM DTT.

Use Terminal Transferase.

TBE buffer:

10x buffer:

108gms Tris base,

55gms Borate,

40ml 0.5M EDTA (pH 8.0).

TAE buffer: 50x:

242 gm Tris base,

57.1 ml Glacial acetic acid,

100ml 0.5M EDTA ph 8.0.

Strength of the Agarose gel for DNA fragments:

0.3% 5-50KB,

0.5% 1-25KBP,

0.8% 700bp- 10KBP,

1% 500bp – 10KBP,

1.5% 200bp- 5KBP,

2% 100bp-2KBP,

2.5% 20bp-1KBP.

20x buffer SSC (NaCl-Na citrate):

174.3 gms NaCl,

88.2 Na citrate,

Make it to 1000ml, pH 8.0.

Hybridization buffer 10X:

0.5M Sodium phosphate (pH 7.2),

7% SDFS,

1mM EDTA,

50% Formamide (ultrapure0,

1x Denhardt sol.

100ug/ml Calf thymus sheared DNA.

Wash buffer:

0. X ssc+0.1%SDS at room Tm,
0.2x SSC +0.1 % SDS at room Tm,
0.2x SSC + 0.1% SDS at 62^o C.

Northern Blotting:

Denaturation buffer:

DMSO- 20ul,
0.1M Na H₂ PO₄ pH7- 4ul,
Deionized glyoxal- 5.9ul,
RNA -10ug.

Mix and incubate at 50^oC for 60 minutes,
Then mix with glyoxal loading buffer;
50% glycerol,
10mM Na H₂PO₄ pH 7.0
0.25% Xylene cyanol,
0.25% Bromophenol blue.

Glyoxal removal buffer:

Submerge the membrane in 20mM Tris-Cl pH 8.0 at 65 ^oC for few minutes.

Preparation of Gel for northern blotting:

RNase is the most dreaded enzyme in all labs. Take precautions to avoid the contamination with RNase.

10mM NaH₂PO₄ pH 7.01 gm of Agarose,

All RNase free.

Running buffer is the same.

Gel loading buffer:

0.25% Bromophenol blue,

0.25% Xylene cyanol,

40% sucrose or

15% Ficoll in water.

Denhardt solution 100 x:

10gm Ficol 400,
10gm PVP,
10gm BSA'
Water to 500ml.

Phage 6M buffer:

50mM Tris-Cl pH 7.8,
8mM Mgcl₂,
100mM NaCl,
0.1% Gelatin.

Phage diluting buffer:

10mM Tris-Cl pH 7.5,
10mM MgCl₂.

Stock EtBr:

10mg/ml.

Depurination solution:

0.25M HCl.

Denaturation buffer:

1.5M NaCl,
0.5M NaOH.

Neutralization buffer:

1M Ammonium acetate,
20mM NaOH,
or
Tris Cl and NaCl.

SDS PAGE buffers:

Stock: 29.2 gms acrylamide,
0.8 gms Bis acrylamide,
Make it to 100ml, store it in cold room.

Separation Gel:

Tris-Cl 10ml (1.5M Tris),
SDS 0.4ml (10% SDS),
Ammonium per sulfate 0.170ml,
TEMED 0.032ml.
Add 16ml of Acrylamide stock solution,
Make it to 40ml.
This is for 10 % gel.

Stacking gel:

Stock acrylamide 1.7ml,
Triscl-6.8 1.25ml (1 M),
SDS 0.1ml,
APS 0.075 ml (10%stck),
TEMED 0.016ml.
Make it to 10ml.

4x sample buffer 10ml:

SDS 2ml,
Tris pH 6.8, 1.2ml (1M Tris),
Glycerol 4ml,
Bromophenol blue 2.5mg,
Coomassie Brilliant Blue 2.5 mg.
B-Mercaptoethanol 2ul/20ul.

Gel Running Buffer:

Tris 15gm,
SDS 5gms,
Glycine 90gms,
Make it to 1liter.

Gel staining soln.:

Methanol 400ml,
Acetic acid 100ml,
Water 500ml,
Coomassie Blue 200 mg/200ml of the above solution.

Destaining solution: same as the above with out the stain.

Sequencing Gel:

TBE buffer (5x):
Tris base 54gms,
Boric acid 27.5 gms,
EDTA 20ml (0.5 M pH 8.0).

Gel loading buffer:

Bromophenol blue 0.25%,
Xylene cyanol 0.25%,
Sucrose 40% or Ficoll 15%

Prepare 5% Polyacrylamide-Urea Gel;

Acrylamide stock 30%,
Urea (7M stock),
TBE (10x) - 5ml,
Acrylamide 30%- 25ml,
Urea -21.gms,
APS- 200ul,
TEMED- 25ul

Western Blot Buffers:

Transfer buffer:
Tris-Glycine SDS containing 20% methanol.

Membrane treatment Buffer:

TBS (50mM Tris *.0, 150mM NaCl) or PBS,
TBST or PBST,
PB=phosphate buffer, (10mM NaPHO₄ (7.0) + 150mM NaCl)
TB=Tris buffer,
T= Tween (.03%),

Blocking buffer: TBST + Skimmed milk 0.5 to 3%; or 0.1%BSA
Washing 2 to 3 times.

Incubation with IgG in PBS (T) or TBS (T) buffer;
Incubation with anti-antibody conjugated with AP in PBS/TBS,
Washing twice to three times;

Alkaline phosphate buffer (pH9.5); 5mM MgCl₂, 50mM Na₂CO₃ (or 100mM NaCl, 100mM Tris-Cl pH 9.5, 5mM MgCl₂.
Then add Nitro BlueTetrazolium (NBT) (100ug/ml, and 5, Bromo 5-chloro 3-Indolyl phosphate (BCIP) 50ug/ml.

Culture media:

Bacterial culture media: LB medium (Luria Broth):

10 gms Bacto Tryptone,
5 gms Bacto-yeast extract,
10 gms NaCl,
Make it to 1 liter.

Phage buffer:

200mM Tris-Cl 7.4,
100mM NaCl,
10mM MgSO₄.

Supplement medium:

For Y1090 cells:
10gm Bacto-Tryptone,
5gm Bacto-yeast extract,
5gm NaCl,
pH 7.5;
50ml LB
0.5 ml 20% maltose,
0.5ml of 1M MgSO₄.

TEP buffer:

100mM Tris-Cl 7.4,
10mM EDTA,
1mM PMSF (10mM PMSF in EtOH),

SM buffer:

50mM Tris-Cl 7.5,
100mM NaCl,
8mM MgSO₄,
0.01% Gelatin.

PCR buffer:

670mM Tris-Cl (.0,
67mM MgCl₂,
1.7mg/ml BSA, 160mM (NH)₄ SO₄.

Yeast culture media:

YP:

10gm Yeast extract,
20gms Bacto-peptone,
Make it to 1 liter.

YPD:

YP + 0.1 volumes of 20% Glucose.

LB for Bacterial transformation or electroporation:

Bacto-Tryptone 2%,
Bacto-yeast extract 0.5%,
NaCl 10mM,
KCl 2.5mM;
Mgcl₂+MgSO₄- 22mM,
PH 6.5;
Mg and Mg So₄ -20mM each,

TFB:

K MES 10mM,
KCl 100mM,
MnCl₂.4h₂O- 45mM,
CaCl₂-2H₂O- 10mM,
H₄COCl₃ (Cobalt chloride)-3mM,
pH- 6.2.

FSB:

K.Acetate 10mM,
Glycerol 10%,
KCl 100mM,
MnCl₂ 45mM,
CaCl₂ 10mM,
HACoCl₃ 3mM. Adjust the pH to 6.2.

Random priming:

10X Hepes pH 6.6
Klenow
3 dNTPs
 $\alpha\alpha\alpha^{32}\text{P}$ CTP (high Ci/mM).

RNA Labeling:

Stock:
10x Transcription buffer,
0.4 Tris-Cl pH 8,
0.8M MgCl₂,
0.5M NaCl,
0.2M Spermidine,
RNA polymerase,
 $\alpha^{32}\text{P}$ -UTP

Purify with G-50 column

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Meanwhile one has to have freshly grown E.coli strains such as Y1089 (lysogenic) or Y1090 (lytic). These have to be grown in LB medium containing MgSO₄ (20mM) and maltose 2%. The density of the cells to be at 0.5 to 0.7 OD.

LB medium:

10 gm Bacto Tryptone,
5 gm Bacto yeast extract,
2% maltose,
20mM MgSO₄,
Adjust the pH to 7.5.

Phage buffer:

200 mM Tris-Cl 7.4,
100 mM NaCl,
10 mM MgSO₄

A list of Solutions and Buffers is unending- the above are some basic solutions used by me.