Molecular Imaging Documentation

Release 0.1

Molecular Imagers

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Contents

CHAPTER 1

General Protocols

0.1

1.1 Common Buffers

1.1.1 Cell Culture

1x PBS

To 800ml ddH2O add:

> 8g NaCl 0.2g KCl 1.44g Na2HPO4 (sodium phosphate dibasic) 0.24g KH2PO4 (monopotassium phosphate)

pH to 7.4, and bring up to 1L with ddH2O. Autoclave to sterilize.

10x PBS

To 800ml ddH2O add:

- 80g NaCl
- 2.0g KCl
- 14.4g Na2HPO4 (sodium phosphate dibasic)
- 2.4g KH2PO4 (monopotassium phosphate)

pH to 7.4, and bring up to 1L with ddH2O. Autoclave to sterilize.

50x TAE

For each litre of solution:

> 242g Tris Base 57.1ml Glacial Acetic Acid 100ml 0.5M EDTA
>
> Mix Tris with stir bar to dissolve in about 600ml of ddH2O. Add the EDTA and Acetic Acid. Bring final volume to 1L with ddH2O. Store at room temperature.

Note: Final (1x) working concentration:

> 0.04M Tris-Acetate 0.001M EDTA

1.1.2 Western Blotting

Lysis Buffer

Reagent	Stock Solution	Volume
ddH_2O	–	7.68 ml
Tris pH 8.0	20 mM	200 ul
NaCl	0.15 M	750 ul
EDTA	2 mM	40 ul
NP40	–	100 ul
Glycerol	–	1000 ul
Na3VO4	1 mM	100 ul

The above recipe is for 10ml. This buffer can be made in bulk and used as a wash buffer, or allotted and frozen for future use. Immediately before use add protease inhibitors at the manufacturers recommended concentration, and phosphatase inhibitors if required.

Laemmli's Buffer, 4x

> 2.4 ml 1 M Tris pH 6.8 (Same as upper gel buffer) 0.8 g SDS stock 4 ml 100% glycerol 0.01% bromophenol blue. Final Concentration is .02% 2.8 ml ddH2O

Before use add 1/10th volume of β-mercaptoethanol

Laemmli's Buffer, 6x

> 1.2g SDS (sodium dodecyl sulfate) 0.01% bromophenol blue 4.7ml glycerol 1.2ml Tris 0.5M pH6.8 2.1ml ddH2O

Before use add 1/8th volume of β-mercaptoethanol

4x Lower Gel Buffer

> 182g Tris-Base (1.5M) 1L dH2O

pH to 8.8; do not overshoot. Use glass pipettes to pH.

4x Upper Gel Buffer

> 30.28g Tris-HCl (0.5M) 500ml dH2O

pH to 6.8, do not overshoot. Use glass pipettes.

10x Running Buffer

> 30.4g Tri-HCl 144.2g Glycine 10g SDS

Dissolve in 1L dH2O. Dilute 1:10 in ddH2O for use as running buffer

10X Transfer Buffer (Towbin Buffer)

> 30.3g Tris-Base 144.15g Glycine 100ml 10% SDS (0.1%)

Bring upto 1L in ddH2O.

1X Transfer Buffer (Towbin Buffer)

> 100ml 10X buffer 200ml methanol 700ml ddH2O

10x TBS

> 302.5g Tris-Base 400g NaCl 18g KCl

Dilute to 5L in dH2O, pH to 7.5 with saturated HCl.

Wash Buffer (TBST)

to 1X TBS ADD:

Standard 0.1% Tween-20

Strong 0.1% Tween-20 + 0.1% NP-40

Extra-Strong 0.1% Tween-20 + 1.5% NP-40

Note: Standard buffer is for most application. Strong/extra strong are only for antibodies with a high degree of non-specificity.

DNA Buffers

6X DNA Loading Buffer

> 6.7 ml of ddH2O 10 mg of bromophenol blue 10 mg of xylene cyanol FF (optional) 3.3ml glycerol

Notes:

> Bromophenol Blue runs at ~300bp (should be added to avoid over-running of gels) Xylene cyanol FF runs at ~4000bp (optional dye) 60 mM EDTA can be added to inhibit DNA-altering enzymes (600ul of 1M EDTA; reduce water appropriately)

Cell Culture Media

2.1 Dulbecco's Modified Eagle Media

DMEM	500 ml	
Glutamine 100x	5 ml	2 mM
FBS	50 ml	10 %
Pen-Strep 100x	5 ml	5 ug/l ??

2.2 Freezing Medium

Complete medium	950 µl	90 %
DMSO	50 µl	10 %

CHAPTER 3

Indices and tables

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