CELL RECIPES

H-50 BUFFER:

Final Concentration	Stock Solution	Volume/Weight of Stock
20 mM HEPES	------	0.477 g
50 mM KCl	2M	2.5 mL
1 mM $MgSO_4$	1M	100 uL
5 mM $NaHCO_3$	------	0.0420 g
10 mM NaCl	5M	200 uL
1 mM NaH_2PO_4	------	0.012 g

pH to 7.0, autoclave or filter sterilize, store at 4˚C

10X MOPS:

200 mM MOPS

50 mM Sodium Acetate

10 mM EDTA

10 mM EGTA

pH=7

HL-5 MEDIA:

For two liters

Yeast Extract	10 grams
Thiotone E. Peptone	15 grams
Proteose Pepton	15 grams
Glucose	20 grams
Na_2HPO_4	0.7 grams
KH_2PO_4	2.4 grams

Add dH_2O to the total volume of 2 L.

pH to 6.5 with HCl/-OH and autoclave

uracil: 40 ug/mL

penicillin + streptomycin: 10 I.U./mL + 10 ug/mL

G418 and/or blasticidin: 10 ug/Ml

LoFlo Media: (Use for fluorescence microscopy to decrease background fluorescence.)

1 mL 1000 X FM Salts 1

1 mL 1000 X FM Salts 2

100 uL 10000 X FM Trace Elements

10 g glucose

0.8709 g K_2HPO_4

5 g Casein Peptone

fill to 1L with dH_2O

pH to 6.5 and filter sterilize

(Autoclaving increases fluorescence.)

Cells should grow ok in this media for a few hours before they are used. If desired, 0.7 g of Oxoid yeast extract can be added. Cells will grow better but there will be more fluorescence.

1000 X FM Salts 1

500 mM NH_4Cl

200 mM $MgCl_2$

10 mM $CaCl_2$

filter sterlilize

or autoclave

1000 X FM Salts 2

50 mM $FeCl_3$

should be yellow

filter sterilize

10000 X Trace Elements

4.84 g $Na_2EDTA\text{-}2H_2O$

2.3 g $ZnSO_4\text{-}7H_2O$

1.11 g H_3BO_4

0.51 g $MnCl_2\text{-}4H_2O$

0.17 g $CoCl_2\text{-}6H_2O$

0.15 g $CuSO_4\text{-}5H_2O$

0.10 g $(NH_4)_6Mo_7O_{24}\text{-}4H_2O$

to 100 mL with dH_2O

pH to 6.5

(blue/purple appearance)

filter sterilize

STARVATION BUFFER*:*

20 mM MES pH 6.8

0.2 mM $CaCl_2$

2 mM $MgSO_4$

Autoclave.

SOC:

100 mL LB + 1 mL 250 mM KCl

0.5 mL 2 M $MgCl_2$

2 mL 1 M glucose

LYSIS BUFFER:

FINAL CONC.	STOCK	VOLUME
5 mM Tris pH 8.0	1 M	0.5 mL
20 mM Sodium PyroPhosphate	200 mM	0.1 mL
5 mM EDTA pH 6.0	0.5 M	1 mL
5 mM EGTA	0.2 M	2.5 mL
0.5% TRITON X-100	20%	2.5 mL
dH_2O	---	---

Distilled water is added until a total volume of 100 mL is reached.

*Add protease inhitors

Store in a -20°C freezer.

WASHING BUFFER:

0.1 mM MES pH 6.8

2.5 mM EGTA

1 mM $MgCl_2$

PHOSPHATE BUFFER: (for centrosome isolation)

14.6 mM KH2PO4	0.198 g
2 mM Na_2HPO_4	0.027 g

Total Volume	100 mL