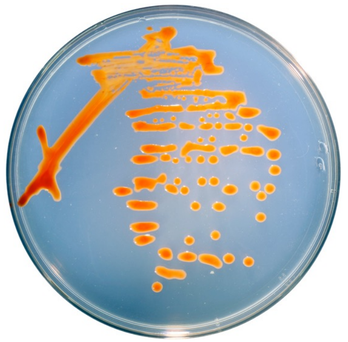
HALOFERAX MEDIA

MARKERS VS MEDIA



Marker	HV-YPC	HV-CA	HV-MIN
pyrE2		+ura	+ura
trpA		+trp	+trp
leuB			+leu
hdrB	+thy	+thy +hypox	+thy +hypox +met +gly +panto

30% SALT WATER (30% SW)

- 4L dH_2O, microwave until hot and stir on hot plate
- 1200g NaCl
- 150g $MgCl_2 \cdot 6H_2O$
- 175g $MgSO_4 \cdot 7H_2O$
- 35g KCl
- 100ml 1M Tris.HCl pH7.5
- Make up to 5L with dH_2O
  - To make 20% SW, use 2L 30% SW and 1L dH_2O

10X YPC (FOR 5 BOTTLES)

- 130ml dH_2O
- 8.5g yeast extract
- 1.7g peptone (Oxoid)
- 1.7g casamino acids
- 3ml 1M KOH (add slowly)
- Make up 170ml with dH_2O

10X YPC (FOR 10 BOTTLES)

- 250ml dH_2O
- 17g yeast extract
- 3.4g peptone (Oxoid)
- 3.4g casamino acids
- 6ml 1M KOH (add slowly)
- Make up 340ml with dH_2O

Allers et al (2004)

HV-YPC BROTH

- Into each 500ml Duran bottle (media grade) measure:
- 300ml 20% SW
  - Or 200ml 30% SW and 100ml dH_2O
- 33ml 10X YPC
- Autoclave for 1 minutes at 121°C (program 1)
- When cool, add 2ml 0.5M CaCl2 and store in dark cupboard

Allers et al (2004)

HV-YPC BROTH – LARGE VOLUMES

	Molarity	1 litre	2 litres	4 litres	8 litres
dH_2O		800 ml	1.600 ml	3.200 ml	6.400 ml
NaCl	2,4641 M	144 g	288 g	576 g	1.152 g
$MgSO_4.7H_2O$	0,0852 M	21 g	42 g	84 g	168 g
$MgCl_2.6H_20$	0,0885 M	18 g	36 g	72 g	144 g
KCl	0,0563 M	4 g	8 g	17 g	34 g
1M Tris pH7.5	0,012 M	12 ml	24 ml	48 ml	96 ml
Yeast extract	0.5% (wt/vol)	5 g	10 g	20 g	40 g
Peptone	0.1% (wt/vol)	1 g	2 g	4 g	8 g
Casamino acids	0.1% (wt/vol)	1 g	2 g	4 g	8 g
Add dH_2O to		1.000 ml	2.000 ml	4.000 ml	8.000 ml
Sterilise in large autoclave on sugar run and leave to cool overnight					
0.5 M $CaCl_2$	*0,003 M*	*6 ml*	*12 ml*	*24 ml*	*48 ml*

HV-YPC AGAR

- Into each 500ml Duran bottle (media grade) measure:
  - 300ml 20% SW
  - 5g agar
- Microwave, for 10 bottles: 20 min on High (100%), 20 min on Medium/High (70%), 2x 10 min on Medium (50%)
- Add 33ml 10X YPC and large stir bar
- Autoclave immediately for 1 minutes at 121°C (program 1)
- Leave in water bath at 60°C to cool

Allers et al (2004)

HV-YPC AGAR PLATES

- Mark plates on side of lid with thick **BLUE** stripe
- Use thin stripe for optional additives and write on plate:
  - 3.4ml thymidine (1/100 of 4mg/ml in dH_2O, to 40µg/ml), red
  - 67µl novobiocin (1/500 of 1mg/ml in dH_2O, to 0.2µg/ml), black
  - 67µl mevinolin (1/5000 of 10mg/ml in ethanol, to 2µg/ml), purple
- When cool, add 2ml 0.5M CaCl2 plus additives while stirring
- Pour 9 thick plates of ~37ml per bottle
- Seal 18 plates per bag, in YPC box in cold room, update board

10X CA (FOR 5 BOTTLES)

- 130ml dH_2O
- 8.5g casamino acids
- 3ml 1M KOH (add slowly)
- Make up 170ml with dH_2O

10X CA (FOR 10 BOTTLES)

- 250ml dH_2O
- 17g casamino acids
- 6ml 1M KOH (add slowly)
- Make up 340ml with dH_2O

Allers et al (2004)

HV-CA BROTH

- Into each 500ml Duran bottle (media grade) measure:
  - 200ml 30% SW
  - 10ml 1M Tris.HCl pH7.0
  - 77ml dH_2O
  - 33ml 10X Ca
- Autoclave for 1 minutes at 121°C (program 1)
- When cool, add:
  - 8.5ml Hv-Min Carbon source
  - 4ml Hv-Min salts
  - 666µl 0.5M KPO4 buffer (pH7.0)
  - 435µl thiamine&biotin
- Store in dark cupboard

Allers et al (2004)

HV-CA AGAR

- Into each 500ml Duran bottle (media grade) measure:
  - 300ml 20% SW
  - 5g agar
- Microwave, for 10 bottles: 20 min on High (100%), 20 min on Medium/High (70%), 2x 10 min on Medium (50%)
- Add 33ml 10X Ca and large stir bar
- Autoclave immediately for 1 minutes at 121°C (program 1)
- Leave in water bath at 60°C to cool

Allers et al (2004)

HV-CA AGAR PLATES

- Mark plates on side of lid with thick **RED** stripe
- Use thin stripe for optional additives and write on plate:
  - 3.4ml thymidine&hypoxanthine (1/100 of 4mg/ml ea. in 50 mM NaOH to 40µg/ml), red
  - 1.7ml tryptophan (1/200 of 10mg/ml in dH_2O to 50 µg/ml), brown
  - 340µl uracil (1/1000 of 50mg/ml in DMSO to 50µg/ml), pink
  - 340µl 5-FOA (1/1000 of 50mg/ml in DMSO to 50µg/ml) +67µl uracil (to 10µg/ml), 2x pink
- When cool, add while stirring gently:
  - 2.8ml Hv-Ca salts
  - 666µl 0.5M KPO4 buffer (pH7.0)
  - Plus additives
- Pour 9 thick plates of ~37ml per bottle
- Seal 18 plates per bag, store in Ca box in cold room, update board

HV-MIN BROTH – UREA-BASED

- Into each 500ml Duran bottle (media grade) measure:
  - 200ml 30% SW
  - 10ml 1M Tris.HCl pH7.0
  - 77ml dH_2O
- Autoclave for 1 minutes at 121°C (program 1)
- When cool, add:
  - 8.5ml Hv-Min carbon source
  - 4ml Hv-Min salts (urea)
  - 666µl 0.5M KPO4 buffer (pH7.0)
  - 435µl thiamine&biotin
- Store in dark cupboard

Mevarech & Werczberger (1985) ; Allers et al (2004)

HV-"MIN" BROTH – AMINO ACID-BASED

- Into each 500ml Duran bottle (media grade) measure:
  - 200ml 30% SW
  - 10ml 1M Tris.HCl pH7.0
  - 77ml dH_2O
- Autoclave for 1 minutes at 121°C (program 1)
- When cool, add:
  - 35ml Hv-Min amino acid mix
  - 8.5ml Hv-Min carbon source
  - 2ml 0.5M CaCl2
  - 333µl trace elements
  - 666µl 0.5M KPO4 buffer (pH7.0)
  - 435µl thiamine&biotin
- Store in dark cupboard

Mevarech & Werczberger (1985) ; Allers et al (2004)

HV-MIN AGAR

- Into each 500ml Duran bottle (media grade) measure:
  - 300ml 20% SW
  - 10ml dH_2O
  - 10ml 1M Tris.HCl pH7.0
  - 5g agar
- Microwave, for 10 bottles: 20 min on High (100%), 20 min on Medium/ High (70%), 2x 10 min on Medium (50%)
- Add large stir bar, autoclave immediately for 1 minutes at 121°C
- Leave in water bath at 60°C to cool

Mevarech & Werczberger (1985) ; Allers et al (2004)

HV-MIN AGAR PLATES

- Mark plates on side of lid with thick **GREEN** stripe
- Use thin stripe for optional additives and write on plate:
  - 3.4ml thymidine&hypoxanthine (1/100 of 4mg/ml each in 50 mM NaOH to 40µg/ml), red
    - Plus 1.7ml methionine & glycine & pantothenic acid (1/200 of 10 mg/ml each in dH_2O, to 50µg/ml)
  - 1.7ml tryptophan (1/200 of 10mg/ml in dH_2O to 50 µg/ml), brown
  - 340µl uracil (1/1000 of 50mg/ml in DMSO to 50µg/ml), pink
  - 1.7ml leucine (1/200 of 10mg/ml in dH_2O to 50 µg/ml), green
  - 2.2ml histidine (1/150 of 10mg/ml in dH_2O to 65 µg/ml), blue
  - 1.7ml methionine (1/200 of 10mg/ml in dH_2O to 50 µg/ml), orange
- When cool, add while stirring gently:
  - 8.5ml Hv-Min Carbon Source
  - 4ml Hv-Min salts
  - 666µl 0.5M KPO4 buffer (pH7.0)
  - 435µl thiamine&biotin
  - Plus additives
- Pour 9 thick plates of ~37ml per bottle, seal 18 plates per bag, store in Min box in cold room

80% GLYCEROL 6% SW

- 80ml glycerol
- 20ml 30% SW
- Autoclave for 1 minutes at 121°C (program 1)
- When cool add 200µl 0.5 M CaCl2

18% SW

- 200ml 30% SW
- 133ml dH_2O
- Autoclave for 1 minutes at 121°C (program 1)
- When cool add 2ml 0.5M CaCl2

HV-CA SALTS

- 13ml thiamine&biotin
- 60ml 0.5M $CaCl_2$
- 10ml trace elements
- Store at 4°C

0.5M $CaCl_2$

- 800ml dH_2O
- 73.5g $CaCl_2$
- Make up 1L with dH_2O
- Filter sterilise

HV-MIN SALTS

- 30ml 1M NH_4Cl (sterile)
- 36ml 0.5M $CaCl_2$
- 6ml trace elements
- Store at 4°C

HV-MIN SALTS – UREA

- 30ml 1M urea (sterile)
- 36ml 0.5M $CaCl_2$
- 6ml trace elements
- Store at 4°C

0.5M KPO_4 BUFFER pH7.0

- 61.5ml 1M K_2HPO_4
- 38.5ml 1M KH_2PO_4
- Check pH = 7.0
- Store at 4°C

THIAMINE&BIOTIN

- 12ml thiamine (1mg/ml in dH_2O)
- 7.5ml biotin (0.2mg/ml in dH_2O)
- Store at 4°C

TRACE ELEMENTS

- 250ml dH_2O
  - Add a few drops cHCl
- 90mg $MnCl_2{\cdot}4H_2O$
- 110mg $ZnSO_4{\cdot}7H_2O$
- 575mg $FeSO_4{\cdot}7H_2O$
- 12.5mg $CuSO_4{\cdot}5H_2O$
- Filter sterilise
- Store at 4°C

HV-MIN CARBON SOURCE

- 150ml dH_2O
- 41.7ml 60% DL-lactic acid Na^+salt
- 33.3g succinic acid disodium salt·$6H_2O$
- 6.3ml 80% glycerol
- Check pH = 7.0, make up 250 ml with dH_2O
- Filter sterilise, store at 4°C

AMINO ACID MIX (50mM)

- Weigh out amino acids
  - See table for proportions
- Blend powders in grinder
  - Pulse to avoid overheating
- Store in dark cupboard
- Dissolve 654.14mg mix in 100ml dH_2O for 50mM stock
- Filter sterilise, store at 4°C

AMINO ACID COCKTAIL

Name			MW	50 ml stock (50 mM)	100 ml Cocktail (50 mM)	100 ml Mix (50 mM)	1 litre Mix (50 mM)	5 litre Mix (50 mM)
Alanine	Ala	A	89,09	222.73 mg	10.42 ml	46.42 mg	**464.2 mg**	2.321 g
Cysteine	Cys	C	121,16	302.90 mg	0.69 ml	4.18 mg	**41.8 mg**	0.209 g
Aspartic Acid	Asp	D	133,1	332.75 mg	9.47 ml	63.02 mg	**630.2 mg**	3.151 g
Glutamic Acid	Glu	E	185,2	463.00 mg	9.38 ml	86.86 mg	**868.6 mg**	4.343 g
Phenylalanine	Phe	F	165,19	412.98 mg	3.13 ml	25.85 mg	**258.5 mg**	1.293 g
Glycine	Gly	G	75,07	187.68 mg	8.26 ml	31.00 mg	**310.0 mg**	1.550 g
Histidine	His	H	155,15	387.88 mg	2.08 ml	16.14 mg	**161.4 mg**	0.807 g
Isoleucine	Ile	I	131,17	327.93 mg	3.96 ml	25.97 mg	**259.7 mg**	1.299 g
Lysine	Lys	K	146,19	365.48 mg	2.47 ml	18.05 mg	**180.5 mg**	0.903 g
Leucine	Leu	L	131,17	327.93 mg	8.19 ml	53.71 mg	**537.1 mg**	2.686 g
Methionine	Met	M	149,21	373.03 mg	1.83 ml	13.65 mg	**136.5 mg**	0.683 g
Asparagine	Asn	N	132,12	330.30 mg	2.65 ml	17.51 mg	**175.1 mg**	0.875 g
Proline	Pro	P	115,13	287.83 mg	4.42 ml	25.44 mg	**254.4 mg**	1.272 g
Glutamine	Gln	Q	146,14	365.35 mg	2.61 ml	19.07 mg	**190.7 mg**	0.954 g
Arginine	Arg	R	174,2	435.50 mg	6.65 ml	57.92 mg	**579.2 mg**	2.896 g
Serine	Ser	S	105,09	262.73 mg	5.57 ml	29.27 mg	**292.7 mg**	1.463 g
Threonine	Thr	T	119,12	297.80 mg	6.04 ml	35.97 mg	**359.7 mg**	1.799 g
Valine	Val	V	117,15	292.88 mg	8.54 ml	50.02 mg	**500.2 mg**	2.501 g
Tryptophan	Trp	W	204,23	510.58 mg	0.95 ml	9.70 mg	**97.0 mg**	0.485 g
Tyrosine	Tyr	Y	181,19	452.98 mg	2.69 ml	24.37 mg	**243.7 mg**	1.219 g
					100.00 ml	**654.14 mg**	**6541.4 mg**	**32.71 g**

STOCK SOLUTIONS

0.5M EDTA (TO MAKE 1L @ pH 8.0)

- 186.1g EDTA
- 800ml dH_2O
- Adjust to pH 8.0 with NaOH

  - First with ~ 20g pellets added slowly followed by 5M stock solution

NOTE: EDTA will not go into solution until pH 8.0 is reached

10X TBE (TO MAKE 1L)

- 108 g Tris base
- 55 g Boric acid
- 40 ml 0.5M EDTA pH 8.0
- make up to 1 L with dH_2O
- Autoclave

50X TAE (TO MAKE 1L)

- 242 g Tris Base
- 57.1 ml conc. acetic acid

- 100 ml 0.5M EDTA pH 8.0
- make up to 1 L with dH_2O

5X DNA GEL LOADING DYE

- 2.5 ml 1 M Tris-HCl pH 7.5
- 10 ml 0.5 M EDTA
- 7.5 g Ficoll
- 0.125 g Bromophenol blue
- 0.125 g Xylene cyanol
- make up to 50 ml with SDW, store at RT

DNA LADDER

- 200 µl 1kb ladder (500 µg/ml, without dye)
- 100 µl 10X buffer 4 or CutSmart
- 200 µl 5X loading dye
- 500 µl SDW
- Store at 4°C

3M SODIUM ACETATE (@ pH 5.2)

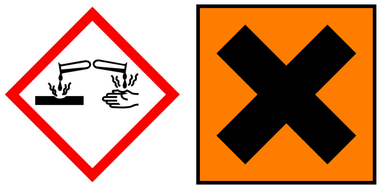
- 61.25 g sodium acetate.$3H_2O$
- 200 ml dH_2O
- Adjust to pH 5.2 with conc. acetic acid

- make up to 250 ml with dH_2O
- Filter sterilise (0.22 µm) and aliquot into 25 ml portions

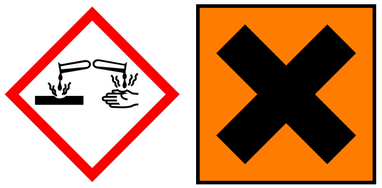
1MM dNTPS

- 20 µl of each dNTP (100 mM stock)
- 1920 µl dH2O
- Aliquot into 100 µl portions, store at –20°C

4X SDS-PAGE LOADING DYE

- 0.5 ml 1 M Tris pH 6.8
- 7.25 ml dH_2O
- 1 ml 20% SDS 
- 10 mg bromophenol blue
- 1.25 ml 80% glycerol

20% SDS

- 200 g SDS 
- 900 ml dH_2O
- Heat and stir SLOWLY until dissolved
- make up to 1 L with dH_2O

NOTE: do not shake as SDS will foam up