

Title JIC Media Preparation lab recipes	
Reference No: QA-MAS-001	Version No: 005

JOHN INNES CENTRE STANDARD OPERATING PROCEDURE	
TITLE: JIC Media Preparation lab recipes	
APPLIES TO STAFF IN: John Innes Centre	
HEALTH & SAFETY INFORMATION INCLUDED: YES	
REFERENCE No: QA-MAS-001	VERSION No: 005
DATE EFFECTIVE: 08.11.11	REVIEW DATE: 07.11.13
AUTHOR: Mary-Anne San Garde	APPROVED BY: H Kieser
QA AUTHORISATION: S Bean	DATE ADDED TO QA DATABASE: 08.11.11

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1 PURPOSE OF PROCEDURE/METHOD AND ITS SCOPE

The purpose of this procedure is to detail recipes used by the JIC Media Preparation laboratory.

2 EQUIPMENT NEEDED

Balance
pH meter
Autoclave

3 STEPS IN PROCEDURE

Wear appropriate personal protective equipment; lab coat, safety glasses and gloves. Observe local safety rules.

MEDIA PROTOCOLS

AT (*Arabidopsis thaliana*)

Formula per 1 litre of de-ionised water

Murashige and Skoog medium (micro and macro elements including vitamins)	4.4g
Sucrose	30.0g
Kinetin	0.05mg
NAA	0.5mg

Adjust pH to 5.8 with 1M NaOH

ATN (JC)

Formula per 1 litre of de-ionised water

Murashige and Skoog medium (micro and macro elements)	4.3g
Sucrose	30.0g
Myo-inositol	100mg
Nicotinic acid	1mg
Pyridoxine	1mg
Thiamine	10mg

Adjust to pH 5.7 with 1M NaOH

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B5

Formula per 1 litre of de-ionised water

Gamborgs B5 Medium Basal salt mixture	3.05g
Gamborgs Vitamin mixture	112mg
Formedium Agar	16g
pH 5.8 with KOH	

B5 Slants add 2% Glucose and 1% formedium agar

BBL 1% Lab M

Formula per 1 litre of de-ionised water

Tryptone Soya Broth	10.0g
NaCl	5.0g
Add per litre	
Lab M agar	10.0g

BBL10mM MgSO₄ 7H₂O 0.6 % Bacto Agar or 0.6% Agarose

Formula per 1 litre of de-ionised water

Tryptone Soya Broth	10.0g
NaCl	5.0g
MgSO ₄ 7H ₂ O	2.465g
Add per litre	
Bacto agar	6.0g
<u>OR</u>	
Agarose	6.0g

BMGY & BMMY

Stock solutions:

500 X B (0.02% Biotin)	Dissolve 20mg biotin in 100ml of water and filter sterilize
10 X GY	MIX 100ML Glycerol with 900ml water and filter sterilize
10 x M	Mix 50ml Methanol with 950ml water and filter sterilize
1M Potassium phosphate buffer pH6	

Combine 132ml of 1M K₂HPO₄ and 868ml 1M KH₂PO₄ **pH to 6** and autoclave.

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To make 1litre BMGY

Yeast extract	10g
Peptone	20g
Yeast nitrogen base	3.8g
Ammonium Sulphate	10g
1M Phosphate Buffer	100ml
500XB	2ml
10XGY	100ml

To make 1 Litre BMMY add 100ml 10 X M instead of 10xGY

BMS

Murashige and Skoog + Vitamins	4.41g
Sucrose	20g
2 4 D	2mg
pH 5.6 with KOH	

BNM

This media is made by making a series of stock solutions

Formula for 1 litre of de-ionised water

200XNOD Major Salts

MgSO ₄	24.4g
KH ₂ PO ₄	13.6g

200XNOD Minor Salts

Formula for 1 litre of de-ionised water

ZnSO ₄ 7H ₂ O	920mg
H ₃ BO ₃	620mg
MnSO ₄ 4H ₂ O	580mg

200XNOD Minor Salts 2

Formula for 1 litre of de-ionised water

NaMoO ₄ 2H ₂ O	50mg
CuSO ₄ 5H ₂ O	5mg

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CoCl₂ 6H₂O 5mg

200XIron-EDTA

Formula is for 1 litre of de-ionised water

Na₂EDTA 3.73g

FeSO₄ 7H₂O 2.78g

To make 1 litre of BNM

CaSO₄ 2H₂O 344mg

MES Buffer 390mg

200XNOD MAJOR SALTS 5ml

200XNOD MINOR SALTS 5ml

200XNOD MINOR SALTS 2 5ml

200XIRON EDTA 5ml

PH to 6 with KOH

For solid media add 11.5g agar

BY 2

Formula per 1 litre of de-ionised water

Murashige and Skoog medium
(micro and macro elements) 4.3g

Sucrose 30.0g

Myo-inositol 100mg

Thiamine 1mg

2,4D 0.2mg

KH₂PO₄ 200mg

Adjust pH to 5.8 with 1M NaOH

De - Naturation

Per 5 litres de-ionised water

Sodium Hydroxide (NaOH) 100g

Sodium Chloride (NaCl) 292.2g

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DNA

Formula per 1 litre of de-ionised water

Difco Nutrient agar	23.0g
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DNB

Formula per 1 litre of de-ionised water

Difco Nutrient broth	8.0g
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DWA

Formula per 1 litre of de-ionised water

Bacto agar	15.0g
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FP (Fahraeus Medium)

This medium is produced by first making a series of stock solutions.

STOCK SOLUTIONS

CaCl ₂ 2H ₂ O	40.0g/l
MgSO ₄ 7H ₂ O	40.0g/l
KH ₂ PO ₄	30.0g/l
Na ₂ HPO ₄ 12 H ₂ O	45.0g/l
FeC ₆ H ₅ O ₇	2.5g/l

Gibson's Trace

Formula per litre

H ₃ BO ₃	2.86g
MnSO ₄ 4H ₂ O	2.03g
ZnSO ₄ 7H ₂ O	220mg
CuSO ₄ 5H ₂ O	80mg
H ₂ MoO ₄	80mg

Use the following amounts of the stock solutions to make 1 litre of medium

CaCl ₂ 2H ₂ O	2.5ml
MgSO ₄ 7H ₂ O	3.0ml

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KH ₂ PO ₄	3.33ml
Na ₂ HPO ₄ 12 H ₂ O	3.33ml
FeC ₆ H ₅ O ₇	2.0ml
Gibson's Trace	1.0ml

PH should be between 6.3 – 6.7

For solid medium add per litre

Lab M agar	5.0g
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For slants add per litre

Lab M agar	10.0g
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FREEZING BROTH

Formula per 1 litre de-ionised water

Tryptone	10.0g
Yeast extract	5.0g
NaCl	5.0g
K ₂ HPO ₄	6.3g
C ₆ H ₅ Na ₃ O ₇ 2H ₂ O	0.45g
MgSO ₄ 7H ₂ O	0.09g
(NH ₄) ₂ SO ₄	0.9g
KH ₂ PO ₄	1.8g
Glycerol	44.0g

Adjust pH to 7.2 with either NaOH or HCl

GB5

This medium is produced with a series of stock solutions
All 7 (Group 1) to 1 litre de-ionised water

Group 1

H ₃ BO ₃	0.62g		
MnSO ₄ 4H ₂ O	2.30g		
ZnSO ₄ 7H ₂ O	0.86g		
KI	Stock of 0.415g/100ml	add	20ml
Na ₂ MoO ₄ 2H ₂ O	Stock of 0.125g/100ml	add	20ml
CuSO ₄ 5H ₂ O	Stock of 0.125g/100ml	add	2ml
CoCl ₂ 6H ₂ O	Stock of 0.125g/100ml	add	2ml

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Group 2

Both Grams per 250ml

FeSO ₄ 7H ₂ O	2.785g
Na ₂ EDTA	3.725g

Group 3

All 3 Grams per 100ml

Nicotinic Acid	0.05g
Pyridoxine	0.05g
Thiamine	0.01g

To make 1litre GB5

CaCl ₂ 2H ₂ O	150mg
KNO ₃	3g
MgSO ₄ 7H ₂ O	500mg
NaH ₂ PO ₄ 2H ₂ O	150mg
(NH ₄) ₂ SO ₄	134mg
Sucrose	30g
MES	500mg
Meso-inositol	100mg

Add 10ml/1lt of seven Group 1 solutions
Add 2.5ml/1lt of Group 2 solutions
Add 1ml 1/lt of Group 3 solutions
PH 5.7 KOH

For Solid media add 8g Bacto Agar

GM GM*

Formula for 1 Litre de-ionised water

Murashige and Skoog (macro and micro elements)	4.3g
Sucrose	10g
Inositol	100mg
Thiamine GM* 0.1mg	1mg

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Pyridoxine 0.5mg

Nicotinic Acid 0.5mg

MES 0.5g

Adjust ph to 5.7 with KOH

If solid media required add 8g agar (GM* 0.9%) 9g/1lt

GM2

Murashidge and skoog + Vitamins 4.41g
 Sucrose 10g
 MES 0.5g
 pH 5.7 with KOH
 Formedium agar 8g

Infiltration medium

Murashidge and skoog minus vitamins 2.165g
 Sucrose 50g
 MES 3mM 0.59g
 pH 5.5

ISP2

Yeast Extract 4g
 Malt Extract 10g
 Glucose 4g
 Difco Bacto Agar 15g

ISP4

Solution 1
 Difco soluble starch 10g make a paste then bring volume to 500ml

Solution 2
 (CaCO₃) 2g
 K₂HPO₄ 1g
 MgSO₄ 1g
 NaCl 1g
 (NH₄)SO₄ 2g

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Trace salt solution	1ml
Distilled water	500ml

Mix two solutions together
pH between 7 and 7.4
Add 20g Difco Bacto agar per 1lt

Trace salt solution per 100ml	
FeSO ₇ H ₂ O	0.1g
MnCl ₂ 4H ₂ O	0.1g
ZnSO ₄ 7H ₂ O	0.1g

KB (Kings B Medium)

Formula per 1 litre de-ionised water

Proteose Peptone	20g
Glycerol	10ml
K ₂ HPO ₄	1.6g

PH to 7.2 with NaOH

For solid	15g Lab M agar
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KNOPS

Calcium Nitrate	0.8g
Magnesium Sulphate	0.25g
Ferrous Sulphate	0.0125g
Ammonium Tartrate	0.5g
Phosphate Buffer	1ml
Formedium agar	8g

To make buffer
Dissolve 25g KH₂PO₄ in 100ml water and titrate with 4M KOH

To make **PROTO** Medium add 66g Mannitol to knops

L broth and L agar (Lennox)

Formula per 1 litre of de-ionised water

Tryptone	10.0g
Yeast Extract	5.0g
NaCl	5.0g
D-Glucose	1.0g

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For solid medium add per litre

Lab M No.1 agar 10.0g

LB (LB – G broth and LB – G agar) (Luria-Bertani) LB*

Formula per 1 litre of de-ionised water

Tryptone 10.0g
 Yeast Extract 5.0g
 NaCl 10.0g

Adjust to pH 7.0 with 1M NaOH.

For solid medium add per litre

Lab M No.1 agar 10.0g

LB* Agar contains 1.5% Lab M Agar 15.0g

LYM

Formula per 1 litre of de-ionised water

Lactose 10.0g
 Yeast Extract 1.0g
 Lab M No. 1 agar 15.0g

M

Make up 4 stock solutions

1litre 20 x Macro Elements

Magnesium Sulphate (MgSO₄ 7H₂O) 7.31g
 Potassium Nitrate (KNO₃) 0.8g
 Potassium Chloride (KCl) 0.65g
 Calcium Nitrate (Ca (NO₃) 4H₂O) 2.88g

500ml 100 X Micro elements

Potassium Phosphate Monobasic (KH₂PO₄) 240mg
 Ethylenediaminetetraacetic acid (NaFeEDTA) 400mg
 Potassium Iodide (KI) 37.5mg
 Manganese Chloride (MnCl₂ 4H₂O) 300mg
 Zinc Sulphate (ZnSO₄ 7H₂O) 132.5mg

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Boric Acid (H_3BO_3)	75mg
Cupric Sulphate ($CuSO_4 \cdot 5H_2O$)	6.5mg
Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)	0.12mg

Mgl

Tryptone	5g
Yeast extract	2.5g
Sodium Chloride	100mg
Mannitol	5g
L-Glutamic acid sodium salt	1g
Potassium Phosphate monobasic (KH_2PO_4)	250mg
Magnesium Sulphate	100mg
Biotin	1ug
pH 7	

Mod FP (Modified FP)

This medium is made with 7 stock solutions per 100ml

1.	Calcium Chloride ($CaCl_2 \cdot 2H_2O$)	13.23g
2.	Magnesium Sulphate ($MgSO_4 \cdot 7H_2O$)	12.32g
3.	Potassium dihydrogen phosphate (KH_2PO_4)	9.53g
4.	Di-sodium hydrogen phosphate (Na_2HPO_4)	11.36g
5.	Ferric Citrate	0.49g
6.	Ammonium Nitrate (NH_4NO_3)	4g

The following 5 components are put into one bottle of 100mls all together making seven bottles.

7.	Manganese Chloride ($MnCl_2 \cdot 4H_2O$)	10mg
	Copper Sulphate ($CuSO_4 \cdot 5H_2O$)	10mg
	Zinc Chloride ($ZnCl_2$)	10mg
	Boric Acid (H_3BO_4)	10mg
	Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)	10mg

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To make MOD FP add 1ml of all seven stock solutions per 1 litre.

pH to 7.5 with NaOH

For solid media add 8g Formedium Agar a litre.

MM (Streptomyces Minimal Medium) 1% IA

Formula per 1 litre of de-ionised water

Asparagine	0.5g
K ₂ HPO ₄	0.5g
MgSO ₄ 7H ₂ O	0.2g
FeSO ₄ 7H ₂ O	0.01g

pH should be between 7.0 and 7.2

Add per litre 10g Iberian Agar

MM (Streptomyces Minimal Medium) 1.5% IA

Formula per 1 litre of de-ionised water

Asparagine	0.5g
K ₂ HPO ₄	0.5g
MgSO ₄ 7H ₂ O	0.2g
FeSO ₄ 7H ₂ O	0.01g

pH should be between 7.0 and 7.2

Add per litre
Iberian Agar 15.0g

MM (Streptomyces Minimal Medium) 1.5% Lab M

Formula per 1 litre of de-ionised water

Asparagine	0.5g
K ₂ HPO ₄	0.5g
MgSO ₄ 7H ₂ O	0.2g
FeSO ₄ 7H ₂ O	0.01g

pH should be between 7.0 and 7.2

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Add per litre

Lab M No 1 agar 15.0g

MM - + 1.5% Lab M (Streptomyces Minimal Medium)

Formula per 1 litre of de-ionised water

(NH ₄) ₂ SO ₄	1.0g
K ₂ HPO ₄	0.5g
MgSO ₄ 7H ₂ O	0.2g
FeSO ₄ 7H ₂ O	0.01g

Adjust pH to 7.0 – 7.2 with 1M NaH₂PO₄

Add per litre

Lab M No 1 agar 15.0g

MS

Formula per 1 litre of de-ionised water

Murashige and Skoog medium (micro and macro elements including vitamins)	4.41g
Sucrose	30.0g

Adjust pH to 5.8 with 1M NaOH

MS Salts (Pauls)

Murashige and skoog salts	4.3g
Sucrose	10g
Phytigel	5g

pH 5.8

MS 0.6%

Formula per 1 litre of de-ionised water

Murashige and Skoog medium (including vitamins)	4.41g
Sucrose	30.0g

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Neutralization

Per 5 litres de-ionised water
Sodium Chloride (NaCl) 876.6g
Tris HCL 302.75g
pH 7.5 with conc HCl approximately 170mls

Nitschs Vitamins

100ml
Thiamine 50mg
Glycine 200mg
Nicotinic Acid 500mg
Pyridoxine 50mg
Folic Acid 50mg
Biotin 5mg

NZ

Formula per 1 litre of de-ionised water

NZ Amine 10.0g
NaCl 5.0g
Casamino Acid 1.0g
MgSO₄ 7H₂O 2.0g

Adjust to pH 7.5 with 5M NaOH.

If solid medium required add per litre

Bacto Agar 15.0g

NZY

NZ Amine 10g
Sodium Chloride (NaCl) 5g
Yeast Extract 5g
Magnesium Sulphate (MgSO₄ 7H₂O) 2g
pH 7.5

For NZY Agar add 1.5% Agar. If top agar is required, use 0.7% Argarose.

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R2

Formula per **800ml** of de-ionised water

Sucrose	103.0g
K ₂ SO ₄	0.25g
MgCl ₂ 6H ₂ O	10.12g
Glucose	10.0g
Casamino acid	0.1g

For solid medium dispense in **80ml** aliquots and add

Bacto Agar	2.2g
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R 5

Formula per 1 litre of de-ionised water

Sucrose	103.0g
K ₂ SO	0.25g
MgCl ₂ 6H ₂ O	10.12g
Glucose	10.0g
Casamino acid	0.1g
Yeast Extract	5.0g
TES	5.73g
Trace elements solution	2.0ml (see under Trace elements for R5 and P)

Adjust to pH 7.0 with 5M NaOH.

For solid medium dispense in **95ml** aliquots and add

Bacto Agar	2.2g
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Regeneration

Formula per 1 litre of de-ionised water

Murashige & Skoog macro and micro elements (MS-)	4.3g
Myo-inositol	100mg
Nitschs vitamins	1ml
Sucrose	20g
Agargel	4g
pH 6 with KOH	

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SD

Yeast Nitrogen Base without Ammonium Sulphate and Amino Acids
(Formedium) 1.9g

(NH₄)SO₄ (Ammonium Sulphate) 5g

Compound dropout as required this amount can vary depending on which selection is needed.

Glucose 20g

Formedium Agar 20g

10 X Dropout for 500ml

L-isoleucine 150mg

L- Valine 750mg

L-Adenine hemisulphate 100mg

L-Arginine 100mg

L-Lycine 150mg

L-Methionine 100mg

L-Phenylalanine 250mg

L- Threonine 1000mg

Tyrosine 150mg

50 X L-Histidine 100mg/100ml

50 X L-Leucine 500mg/100ml

50 X Tryptophan 100mg/100ml

50 X L-Uracil 100mg/100ml

SDS Page

Per 5lt de-ionised water

Tris 15.14g

Glycine 72.07g

10% SDS 50ml

20 X SSC

Per 5 litres de-ionised water

Sodium Chloride (NaCl) 876g

Tri-sodium citrate 441.15g

pH 7.2 with either NaOH or Acetic Acid

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SF + M

Formula per 1 litre of tap water

Soya Flour	20.0g
Mannitol	20.0g
Lab M No1 agar	20.0g

SM

Formula per 1 litre of de-ionised water

NaCl	5.8g
MgSO ₄ 7H ₂ O	2.0g
Gelatin	0.1g
Tris	50ml (1M Tris pH 7.5)

SOC

Formula per 1 litre of de-ionised water

Tryptone	20.0g
Yeast Extract	5.0g
NaCl	0.58g
KCl	0.19g
MgCl ₂	2.03g
MgSO ₄ 7H ₂ O	2.46g
Glucose	3.6g

SMM (Supplemented Minimal Medium)

Formula per 1 litre of de-ionised water

Casamino Acid	2.0g
TES	5.73g

Adjust to pH 7.2 with 5M NaOH

SMMS 1% Lab M

Formula per 1 litre of de-ionised water

Casamino Acid	2.0g
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TES 5.73g

Adjust to pH 7.0 with 5M NaOH

Add per litre

Lab M agar 10.0g

SMMS 1.5% Lab M

Formula per 1 litre of de-ionised water

Casamino Acid 2.0g

TES 5.73g

Adjust to pH 7.0 with 5M NaOH

Add per litre

Lab M agar 15.0g

SMMS 1.5% IA

Formula per 1 litre of de-ionised water

Casamino Acid 2.0g

TES 5.73g

Adjust to pH 7.0 with 5M NaOH

Add per litre

Iberian agar 15.0g

SNA (SOFT NUTRIENT AGAR)

Formula per 1 litre of de-ionised water

Nutrient Broth 8g

Agar 7g

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SUPER YEME

Formula per 1 litre of de-ionised water

Yeast Extract	3.0g
Peptone	5.0g
Malt Extract	3.0g
Glucose	10.0g
Sucrose	340.0g
MgCl ₂ 6H ₂ O	1.1g
Glycine	5.0g
L-Proline	0.075g
L-Arginine	0.075g
L-Histidine	0.1g
Uracil	0.015g
L-Cystine	0.075g

50 x TAE

Per 1litre de-ionised water

Tris	242g
EDTA	37.2g

pH 7.7 with Acetic Acid approx 70 to 80mls

10 X TBE

Per 5 litres de-ionised Water

Tris HCl	540g
Boric Acid	275g
EDTA	46.5g

20 X TBS

Per 1lt de-ionised water

Tris	121g
Sodium Chloride	232g

pH 7.4 with conc HCl approx 65mls

10 X TE

Per 2lt de-ionised water

Tris	24.22g
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Reference No: QA-MAS-001	Version No: 005

EDTA 7.45g
pH 8

TERRIFIC BROTH

The amounts given are per **900ml** de-ionised water.

Tryptone	12.0g
Yeast Extract	24.0g
Glycerol	4.0ml

Dispense in 90ml aliquots.

TRACE ELEMENTS for R5 and P

Formula per 1 litre of de-ionised water

ZnCl ₂	40mg
FeCl ₂ 6H ₂ O	200mg
CuCl ₂ 2H ₂ O	10mg
MnCl ₂ 4H ₂ O	10mg
Na ₂ B ₄ O ₇ 10H ₂ O	10mg
(NH ₄) ₆ Mo ₇ O ₂₄ 4H ₂ O	10mg

TSA

Formula per 1 litre of de-ionised water

Sucrose	10g
Tryptone	10g
Glutamic Acid Sodium Salt	1g
Formedium Agar	15g

pH 6

TY

Formula per 1 litre of de-ionised water

Tryptone	5.0g
Yeast Extract	3.0g
CaCl ₂ 6H ₂ O	1.32g

If solid medium is required add per litre

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Lab M No.1 agar 10.0g

V8

0.5lt V8 Vegetable Juice (Cambells)

0.5lt De-ionised Water

25g Formedium Agar

Water H₂O Agar

Formula per 1litre of de-ionised Water

30g Agar

YEME 34%

Formula is for 1litre of de-ionised water

Yeast extract 3.0g

Peptone 5.00g

Malt extract 3.00g

Glucose 10g

Sucrose 340g

YEB

Peptone 5g

Yeast extract 1g

Beef Extract 5g

Sucrose 5g

Magnesium Sulphate 5g

Formula per 1 litre of de-ionised water

YEP

Formula per 1 litre of de-ionised water

Peptone 10g

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Yeast Extract	10g
NaCl	5g

YMA

Formula per 1 litre of de-ionised water

Mannitol	7g
Yeast Extract	2g
K ₂ HPO ₄	0.2g
MgSO ₄	0.2g
Lab M Agar	10g

2 x YT

Formula per 1 litre of de-ionised water

Tryptone	16.0g
Yeast Extract	10.0g
NaCl	5.0g

Adjust pH to 7.4 with 5M NaOH

If solid medium is required add 10g Lab M agar

YPAD

Formula per 1 litre of de-ionised water

Yeast Extract	10g
Peptone	20g
Glucose	20g
Adenine	20mg

4 RISK STATEMENT

Low risk provided all appropriate precautions detailed in this procedure and relevant local safety rules are followed.

All individuals using this procedure will be given appropriate information, instruction and training in the risks and precautions necessary, including the use of any personal protective equipment required.

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Reference No: QA-MAS-001	Version No: 005

5 DOCUMENTATION

Link to: JIC Chemical Tables:

http://intranet/infoserv/support/QualityAssurance/Chemical_Tables_SOPs.htm

Link to: Good Laboratory Practice in the Use of Chemicals:

http://intranet/infoserv/support/Safety/Chemical/GLP_Chems.htm

Link to Laboratory Waste Disposal:

<http://intranet/infoserv/Support/Safety/Waste/index.htm>

For specific risk assessment information and R and S phrases information please refer to Risk Assessment on the intranet: (<http://intranet/infoserv/support/Safety/Risk/index.htm>).

6 RELATED PROCEDURES

None

7 NOTES

None

8 APPENDICES

None

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