Methods S1. Media and purine overlay formulations used for growth and assessments of purine utilization, related to STAR Methods.

Media formulations

METHOD: 11E rich medium with glucose + maltose (2X)

Component	**Amount**	**[1× Medium]**	**Notes/Comments**
Tap distilled H_2O	200 ml		
1 M $K_xH_xPO_4$, pH 7.2	30 ml	60 mM	
Tryptone	8.0 g		BD 211921
Yeast Extract	4.0 g		Fisher Sci. BP1422
Meat Extract	4.0 g		HiMedia RM003
Tween 80 (25% soln.)	0.5 ml		>Optional
0.025% Resazurin	1.0 ml		
NaAcetate·$3H_2O$	1.7 g	25 mM	
D-(+)-Glucose FW = 180.16	1.35 g	15 mM	
Maltose FW = 342.3	1.71	10 mM	
[L-Lysine·2HCl FW = 219.11	2.75 g]	25 mM	>Optional; as required
$NaHCO_3$ FW = 84.0	1.05 g	25 mM	
Histidine-Hematin soln.	0.25 ml		
ATCC Vitamin soln. (MD-VS)	2.5 ml		
Adjust pH to ~7.2 + ~350 µl 10N NaOH			
5 mM $FeSO_4·7H_2O$	0.25 ml	2.5 µM	
Adjust volume = **250 ml**			
L-cysteine	0.25 g		

Immediately filter sterilize medium; place in anaerobic glove chamber, loosen cap overnight, then retighten cap.

1x medium: Combine with equal volume sterile anaerobic water.
Plates: Combine with equal volume 2.4% agar.

METHOD: #23B medium (2×) for liquid cultures

Component	Amount	[Medium]	Notes/Comments
Milli-Q H_2O	400 ml	**per 1×**	
1 M K_xPO_4, pH 7.2	50 ml	50 mM	
0.025% Resazurin	2.0 ml		
Yeast Extract	1.0 g	0.10%	
NaAcetate·$3H_2O$ FW 136	3.4 g	25 mM	
NaCl FW 58.44	1.46 g	25 mM	
0.5 M K_2SO_4	1.0 ml	0.5 mM	
1.0 M $MgCl_2·6H_2O$	1.0 ml	1 mM	
1.0 M $CaCl_2·2H_2O$	0.5 ml	0.5 mM	
$NaHCO_3$ FW = 84.0	1.68 g	20 mM	
"2x" Vit. K_1 + K_3 soln.	125 µl		
ATCC Vitamin Mixture (MD – VS)	10 ml		
Adjust pH @ 7.0 ~600 ul 10N NaOH → pH 7.23			
"50×" Trace Minerals	5.0 ml		
5 mM $FeSO_4·7H_2O$	1.0 ml	5 µM	
Add Milli-Q H_2O to:	500 ml		
L-cysteine·HCl FW 174.63	0.88 g	5 mM	

Immediately filter sterilize medium; place in anaerobic glove chamber, loosen cap overnight, then retighten cap.

To prepare cultures combine 1:1 with anerobic, sterile water. Add stocks of carbohydrates and NH_4Cl as required.

To prepare cultures for purine catabolism, introduce purine (e.g., 12 mg uric acid) into 125 × 16 mm Hungate tubes, transfer into the anaerobic chamber and allow deoxygenation (overnight). Then add 5 ml Milli-Q purified anaerobic water, seal tubes and autoclave (121°C/20 minutes). Vortex vigorously when removed from the autoclave to disperse substrate, allow to cool, then introduce 5 ml sterile medium/tube by syringe.

METHOD: #26B medium for plates

Component	Amount	[Medium]	Notes/Comments
Milli-Q H_2O	800 ml		[Plates = Medium × ½]
1 M $K_xH_xPO_4$, pH 7.2	50 ml	50 mM	25 mM
0.025% Resazurin	2.0 ml		
Tricine FW 179.2	0.72 g	4 mM	2 mM
Yeast Extract	2.0 g	0.20%	0.10%
NaAcetate·$3H_2O$ FW 136	3.4 g	25 mM	12.5 mM
NaCl FW 58.44	1.46 g	25 mM	12.5 mM
0.5 M K_2SO_4	1.0 ml	0.5 mM	0.25 mM
1.0 M $MgCl_2·6H_2O$	1.0 ml	1 mM	0.50 mM
1.0 M $CaCl_2·2H_2O$	0.5 ml	0.5 mM	0.25 mM
$NaHCO_3$ FW = 84.0	1.68 g	20 mM	10 mM
"2x" Vit. K_1 + K_3 soln.	126 µl		
ATCC Vitamin Mixture (MD – VS)	10 ml		
Adjust pH To 7.3 (@6.99) ~ 600 ul 10N NaOH →pH 7.27			
Add Milli-Q water to:	1000 ml		
"500×" TM #4	2.0 ml		
5 mM $FeSO_4·7H_2O$	1.0 ml	5 µM	2.5 µM
5 mM Mo	2.0 ml	10 µM	5.0 µM
1 mM Se	1.0 ml	1 µM	0.5 µM
L-cysteine·HCl FW 175.64	0.88 g	5 mM	2.5 mM

Immediately filter sterilize medium; place in anaerobic glove chamber, loosen cap overnight, then retighten cap.

Combine in the ratio of 1:1 with sterile, molten 2.4% Bacto Agar.

METHOD: #26B-All medium for monolayer plates containing allantoin

Component	Amount	[Medium]	Notes/Comments
Milli-Q H_2O	440 ml		[Plates = Medium × 3/4]
1 M $K_xH_xPO_4$, pH 7.2	16.7 ml	33.4 mM	25 mM in plates
0.025% Resazurin	0.67 ml		
Tricine FW 179.1	0.24 g	2.68 mM	2 mM
Yeast Extract	0.67 g	0.134%	0.10%
NaAcetate·$3H_2O$ FW 136	1.14 g	16.7 mM	(optional)
NaCl FW 58.44	0.49 g	16.7 mM	12.6 mM
0.5 M K_2SO_4	0.333 ml	0.333 mM	0.25 mM
1.0 M $MgCl_2·6H_2O$	0.333 ml	0.667 mM	0.50 mM
1.0 M $CaCl_2·2H_2O$	0.167 ml	0.334 mM	0.25 mM
$NaHCO_3$ FW = 84.0	0.56 g	13.33 mM	10 mM
"2x" Vit. K_1 + K_3 soln.	42 ul		
Allantoin FW 158.1	4.74 g	60 mM	45 mM (Alfa Aesar A15571.30 lot 10199372, 98%)
ATCC Vitamin Mixture (MD – VS)	3.33 ml		
Adjust pH to 7.3 (@ 7.06): add ~ 260 µl 10N NaOH, pH --> 7.27 After pH adjustment heat to ~50°C with mixing to fully dissolve Allantoin (microwave 3×30 seconds).			
Add Milli-Q water to:	500 ml		
"500×" TM #4	0.667 ml		
5 mM $FeSO_4·7H_2O$	0.333 ml	3.33 µM	2.5 µM
5 mM $NaMoO_4·2H_2O$	0.667 ml	6.67 µM	5.0 µM
1 mM Na_2SeO_3	0.333 ml	0.67 µM	0.5 µM
L-cysteine·HCl FW 175.64	0.30 g	3.42 mM	2.6 mM
Immediately filter sterilize medium; place in anaerobic glove chamber, loosen cap overnight, then retighten cap. Combine in the ratio of 1:3 with sterile, molten 4.8% Bacto Agar. Use promptly; upon prolonged storage some allantoin will recrystallize.			

Stock solutions:

1M Potassium Phosphate Buffer, pH 7.2
121.9 g K_2HPO_4 (anhydrous, MW = 174.2) + 40.8 g KH_2PO_4 (anhydrous MW = 136.1) dissolved in ~900 ml Milli-Q H_2O. Check pH (~7.2), adjust pH to 7.2, adjust volume to 1.0 l.

5 mM $FeSO_4 \cdot 7H_2O$ (Fisher Scientific #I146-500)
0.139 g in 100 ml H_2SO_4-acidified MilliQ water (acidify with 4 drops H_2SO_4).

5 mM $Na_2MoO_4 \cdot 2H_2O$ (MP Biomedicals #194863)
0.121 g in 100 ml H_2SO_4-acidified MilliQ water.

1 mM Na_2SeO_3 (Acros Organics #200730250)
0.0173 g in 100 ml H_2SO_4-acidified MilliQ water.

"2×" Vitamins K_1 + K_3 solution
0.1 ml viscous liquid vitamin K_1 (Chem-Impex Intl., #00768) dissolved into 10 ml 100% ethanol, + 20 mg vitamin K_3 (Sigma M5625), filter sterilize, store at -20°C.

Histidine-Hematin solution

1. 0.2 M histidine, pH 8.0: Add 2.1 g histidine-HCl·H_2O (Sigma #H7875) into 40 ml distilled H_2O. Adjust pH from ~4 to 8.0 with 10N NaOH (just over 1 ml, histidine should dissolve). Adjust the final volume to 50 ml with distilled H_2O.
2. Mix 12 mg hematin (Sigma #H3281) + 10 ml 0.2 M histidine, pH 8.0. Dissolve by vigorous shaking for several hours. Filter sterilize, store aliquots at -20°C.

"50×" Trace Minerals (TM)
1000 ml MilliQ water, acidified with 5 drops H_2SO_4. Store under N_2, refrigerated.

Component	FW	g	[Stock]	Medium (1:200-diluted)
$MnCl_2 \cdot 4H_2O$	197.9	0.2969	1500 µM	7.5 µM
$ZnCl_2$	136.3	0.0682	500	2.5
$CoCl_2 \cdot 6H_2O$	237.9	0.0476	200	1.0
$Na_2MoO4 \cdot 2H_2O$	242.0	0.0121	50	0.25
Na_2SeO_3	172.9	0.0086	50	0.25
$NiCl_2 \cdot 6H_2O$	237.7	0.0594	250	1.25
$Na_2WO_4 \cdot 2H_2O$	329.9	0.0165	50	0.25

Trace Minerals (TM) #4

1000 ml MilliQ water, acidified with 5 drops H_2SO_4. Store under N_2, refrigerated.

Component	FW	g	5000×[Stock]	500×[Stock]	Medium (1:500-diluted)
$MnCl_2 \cdot 4H_2O$	197.9	0.0990	500 µM	50 µM	100 nM
$ZnCl_2$	136.3	0.0682	500	50	100
$CoCl_2 \cdot 6H_2O$	237.9	0.0595	250	25	50
$NiCl_2 \cdot 6H_2O$	237.7	0.1188	500	50	100
$Na_2WO_4 \cdot 2H_2O$	329.9	0.0824	250	25	50

Preparation of top layer overlays for bilayer plates:

Adenine top layer, ~7 ml/plate

20 ml tap distilled H_2O plus:

1.0 ml of 1M stock potassium phosphate buffer, pH 7.2
0.55 g Adenine (Alfa Aesar A14906, 99%)
pH measured at 7.2
0.56 g Bacto Agar (in 40 ml, = 1.4%)

Hypoxanthine top layer, ~7 ml/plate

20 ml tdH_2O plus:

1.0 ml of 1M stock potassium phosphate buffer, pH 7.2
0.52 g Hypoxanthine (Thermo Scientific A11481.06, 99%)
pH measured at 7.2
0.56 g Bacto Agar (in 40 ml, = 1.4%)

Uric Acid top layer, ~7 ml/plate

20 ml tap distilled H_2O plus:

1.0 ml of 1M stock potassium phosphate buffer, pH 7.2
0.48 g Uric Acid (Sigma-Aldrich U0881-100G Pcode 102408543, ≥99%)
pH measured at 7.0
0.56 g Bacto Agar (in 40 ml, = 1.4%)

Autoclave 20 min./121°C, then mix rapidly while cooling to evenly disperse purine. To prepare overlay: under anaerobic conditions add 20 ml medium 26B, mix well and transfer 7 ml onto base layer medium. Mix well between each addition to keep purine suspension uniform.