ATCC Medium: 2871 Chloracidobacterium thermophilum Midnight Medium (CTM-Medium)

1. Preparation of 1 liter CTM-Medium

The CTM-Medium consists of two parts. Part one is autoclavable and called CTM-Medium basis. Part two is named mixed solution and contains non-autoclavable components. Solid CTM-Medium contains 1% thre times washed Bactoagar and is added to the CTM-Medium basis prior autoclaving.

a. Fill medium bottle with
   i. Solution 1 : 20ml
   ii. Solution 2 : 3ml
   iii. Solution 3 : 2ml
   iv. Solution 4 : 2.5ml
   v. HEPES buffer : 2.4g
b. Add ddH_2O close to 1 liter
c. Adjust pH to 7.0 with 2M KOH
d. Ad ddH_2O to 1 liter
e. Autoclave medium 40 min at 121˚C
f. Seal medium bottle immediately after autoclaving in order to avoid oxygenation
g. Let the medium cool down to around 70˚C
h. Add mixed solution to the CTM basis for completing the CTM-Medium
i. Keep CTM-Medium tightly sealed and between 50-70˚C prior usage
j. It is strongly recommended to use up the CTM-Medium as soon as possible after preparation!!!
k. Pour CTM-Medium into flasks, bottles or similar and leave a quarter air headspace and seal them
l. Put solid medium containing vessels in an air tight container, establish a 10% $CO_2$and 10% H_2 nitrogen balanced atmosphere and keep the container sealed. (optional method)

2. Preparation of stock solutions

All stock solutions are autoclavable and should be autoclaved before long term storage at 4-8˚C. Exceptions are stated. ddH_2O is used for all solutions if not mentioned otherwise.

a. Solution 1 (50x stock) components and amounts per liter

i. $MgSO_4$ * 7 H_2O : 3.75g
ii. $CaCl_2$ * 2H_2O : 1.80g
iii. Citric acid : 0.30g
iv. Na-EDTA, pH8, 0.25M : 0.60ml
v. Trace metal solution : 50.0ml

b. Solution 2 components and amounts per liter

i. K_2HPO_4 : 15.3g

c. Solution 3 components and amounts per liter

i. Ferric(NH_4)citrate : 12.0g

d. Solution 4 components and amounts per liter

i. 2-Oxoglutarate : 14.6g

e. Solution 5 components and amounts per 100 ml

i. Bacto peptone : 10.0g

f. Solution 6 components and amounts per 400 ml

i. The 20 proteinogenic AA: 100mg of each AA

g. Trace metal solution components and amounts per liter

i. H_3BO_3 : 2.86g
ii. $MnCl_2$ * 4H_2O : 1.81g
iii. $ZnSO_4$ *7H_2O : 0.222g
iv. Na2MoO_4 * 2H_2O : 0.39g
v. $CuSO_4$ * 5H_2O : 0.079g
vi. Co(NO3)2 * 6H_2O : 0.0494g

h. Vitamin solution A (1000x stock) components and amounts per 100 ml

Dissolve the vitamins in 10mM phosphate buffer, pH7.2

Titrate with NaOH until vitamins are dissolved, filter sterilize afterwards, and freeze in appropriate aliquots until usage

i.	Biotin	:	10mg
ii.	Riboflavin	:	10mg
iii.	Thiamine HCl	:	100mg
iv.	Thiamine pyrophosphate	:	100mg
v.	L-ascorbic acid	:	100mg
vi.	D-Ca-pantothenate	:	100mg
vii.	Folic acid	:	100mg
viii.	Nicotinamide	:	100mg
ix.	Nicotinic acid	:	100mg
x.	4-Aminobenzoic acid	:	100mg
xi.	Pyridoxine HCl	:	100mg
xii.	Lipoic acid	:	100mg
xiii.	NAD	:	100mg

i. Vitamin solution B (1000x stock) components and amounts per 100 ml

Dissolve the vitamin B_{12} in water

Titrate with HCl until vitamin B_{12} is dissolved, filter sterilize afterwards, and freeze in appropriate aliquots until usage

i.	Cyanocobalamin (B_{12})	:	100mg

j. Mixed solution for 1 liter CTM-Medium completion after autoclaving

Always prepare freshly! Mix the following components and add the filter sterilized mixture to the autoclaved CTM-Medium basis

i.	$NaHCO_3$	:	0.625g
ii.	Na-Thioglycolate	:	0.125g
iii.	Solution 5	:	0.5ml
iv.	Solution 6	:	10ml
v.	Vitamin solution A	:	0.25ml
vi.	Vitamin solution B	:	0.25ml
vii.	Add ddH2O to final a volume of 30 ml		