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Auxenochlorella ApM1/ApRM1 media recipes and stocks V.2

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We use this protocol and it's working

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Abstract

This protocol will make 1 L of ApM1 or ApRM1 medium, optimized for heterotrophic growth of *Auxenochlorella protothecoides*. This is high phosphate buffered medium that prevents excessive acidification. Use the attached spreadsheet to adjust masses and volumes as required Auxenochlorella protothecoides me... 114.6KB . The recipe is adapted from US Patent US20140178950A1 (pg. 28), US Patent US8927522B2 (pg. 37) and US Patent US12037630B2 (pg. 18 & 20).

Guidelines

Add phosphate salts to water; do not add water to phosphate salts or they may form an insoluble slag
Ammonium sulfate concentration is 7.5 mM, **ammonium ion concentration is 15 mM**
Ammonium sulfate is added post-sterilization to avoid precipitation
Sugar is added post-sterilization to avoid caramelization
Vitamins and antibiotics are added post-sterilization to avoid degradation

Materials

1 L beaker
1 L autoclavable media storage bottle
2 L Erlenmeyer flask
Milli-Q water
potassium phosphate dibasic anhydrous (Fisher P288-500)
sodium phosphate monobasic monohydrate (Fisher S369-1)
magnesium sulfate heptahydrate (Fisher M63-500)
citric acid (Fisher BP339-500)
calcium chloride dihydrate (Fisher C79-500)
agar (Fisher (BP1423-500)
agarose (Fisher BP1356-500)
yeast extract (Fisher BP9727-500)
ammonium sulfate (Fisher A702-500)
glucose (Fisher D19-212)
thiamine-HCl (Sigma T1270-25G)
sucrose (Fisher S2-500)
melibiose (CHEM-IMPEX 00808)
G418-sulfate (VWR E859-5G)
hygromycin B (VWR K547-20ML)
copper sulfate pentahydrate (Fisher BP346-500)
boric acid (Sigma B9645-500G)
zinc sulfate heptahydrate (Sigma Z0251-500G)
manganese chloride tetrahydrate (Sigma M3634-100G)
sodium molybdate dihydrate (Sigma M1651-100G)
iron sulfate heptahydrate (MP Biochemicals 194663)

Fill beaker to about 700 mL with MilliQ water

1 Add magnetic stir bar and spin to mix

Add ingredients

2 4.2 g potassium phosphate dibasic anhydrous (Fisher P288-500)
[M] 24.1 millimolar (mM)
3.57 g sodium phosphate monobasic monohydrate (Fisher S369-1)
[M] 25.9 millimolar (mM)
240 mg magnesium sulfate heptahydrate (Fisher M63-500)
[M] 974 micromolar (µM)
250 mg citric acid (Fisher BP339-500) [M] 1.3 millimolar (mM)
1.7 mL 100 mM calcium chloride dihydrate (Fisher C79-500)
[M] 170 micromolar (µM)
10 mL 100X Ap trace element solution

Optional for solid media
15 g agar (Fisher (BP1423-500) [M] 1.5 Mass / % volume
OR
8 g agarose (Fisher BP1356-500) [M] 0.8 Mass / % volume ***(use agarose for minus thiamine selection)***

Optional for rich media (ApRM1)
4 g yeast extract (Fisher BP9727-500) [M] 0.4 Mass / % volume

Adjust volume to 909 mL with MilliQ water and autoclave 55m

3 Autoclave 121 °C 00:55:00 1.24 Bar 55m

Post-sterilization additions

4 Cool to 50 °C in water bath

50 mL 150 mM ammonium sulfate (Fisher A702-500) [M] 7.5 millimolar (mM)

40 mL 50% glucose (Fisher D19-212) [M] 2 Mass / % volume

1 mL 2 mM thiamine-HCl (Sigma T1270-25G) [M] 2 micromolar (µM)

Optional for minus-thiamine media - don't add thiamine-HCl

Optional for sucrose media

40 mL 50% sucrose (Fisher S2-500) [M] 2 Mass / % volume

Optional for melibiose media

40 mL 50% melibiose (CHEM-IMPEX 00808) [M] 2 Mass / % volume

Optional for G418 media

1 mL 100 mg/mL G418-sulfate (VWR E859-5G) [M] 100 Mass Percent

Optional for hygromycin B media

6 mL 50 mg/ mL hygromycin B (VWR K547-20ML) [M] 300 Mass Percent

Storage

5 Store liquid medium without sugar, thiamine or antibiotics at Room temperature or 4 °C

Store liquid medium with sugar, thiamine or antibiotics at 4 °C

Store solid medium with sugar, thiamine or antibiotics at 4 °C

Stock solutions

6 **100X Ap trace element solution (1 L)**

2.743 g citric acid (Fisher BP339-500) [M] 14.3 millimolar (mM)

11 mg copper sulfate pentahydrate (Fisher BP346-500) [M] 44.1 micromolar (µM)

330 mg boric acid (Sigma B9645-500G) [M] 340.5 micromolar (µM)

1.4 g zinc sulfate heptahydrate (Sigma Z0251-500G) [M] 4.89 millimolar (mM)

948.5 mg manganese chloride tetrahydrate (Sigma M3634-100G)
[M] 4.79 millimolar (mM)
3.9 mg sodium molybdate dihydrate (Sigma M1651-100G) [M] 161 micromolar (µM)
110 mg iron sulfate heptahydrate (MP Biochemicals 194663)
[M] 396 micromolar (µM)
Add citric acid first, then salts to 700 mL of MilliQ water with a magnetic stir bar and mix to dissolve
Adjust volume to 1 L
Store at 4 °C

100 mM calcium chloride (1 L)

14.701 g calcium chloride dihydrate (Fisher C79-500)
Dissolve in 700 mL MilliQ water with a magnetic stir bar
Adjust volume to 1 L
Store at 4 °C

150 mM ammonium sulfate (1 L)

19.82 g ammonium sulfate (Fisher A702-500) [M] 150 millimolar (mM)
Dissolve in 700 mL MilliQ water with a magnetic stir bar
Adjust volume to 1 L
Filter sterilize into a sterile bottle with a bottle top filter (Fisherbrand FB12566510)
Store at Room temperature

2 mM thiamine hydrochloride (1L)

675 mg Sigma (T1270-25G) [M] 2 millimolar (mM)
Dissolve in 700 mL MilliQ water with a magnetic stir bar
Adjust volume to 1 L
Filter sterilize into a sterile bottle with a bottle top filter (Fisherbrand FB12566510)
Store at 4 °C

50% glucose (1L)

500 g (Fisher D19-212) [M] 50 Mass / % volume
Slowly add to 400 mL MilliQ water with a magnetic stir bar, mix with heat until fully dissolved
Adjust volume to 1 L
Filter sterilize into a sterile bottle with a bottle top filter (Fisherbrand FB12566510)
Store at Room temperature

50% sucrose (1L)

500 g (Fisher S2-500) [M] 50 Mass / % volume

Slowly add to 400 mL MilliQ water with a magnetic stir bar, mix with heat until fully dissolved

Adjust volume to 1 L

Filter sterilize into a sterile bottle with a bottle top filter (Fisherbrand FB12566510)

Store at Room temperature

50% melibiose (500 mL)

250 g melibiose monohydrate (CHEM-IMPEX 00808) [M] 50 Mass / % volume

Slowly add to 200 mL MilliQ water with a magnetic stir bar, mix with heat until fully dissolved

Adjust volume to 500 mL

Filter sterilize into a sterile bottle with a bottle top filter (Fisherbrand FB12566510)

Store at Room temperature

100 mg/mL G418 sulfate (10 mL)

5 g G418 Sulfate (VWR E859-5G)

Dissolve in 40 mL MilliQ water

Adjust volume to 50 mL

Filter sterilize with a 0.22 µm syringe filter (MIliporeSigma SLGVR33RB)

Aliquot 1 mL into sterile eppendorf tubes

Store aliquots at -20 °C

Protocol references

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