

Methanococcus agar medium (McCas or N-free)

Make sure to have Petri dishes in the anaerobic chamber at least 1 day ahead of time to allow oxygen to diffuse from them.

	<u>200 ml:</u>	<u>500 ml:</u>	<u>600 ml</u>
<u>Combine:</u>			
H ₂ O	100 ml	250 ml	300 ml
General Salts solution (McCas)	100 ml	250 ml	300 ml

OR N-free general salts (N-free)

NaHCO ₃	0.4 g	1 g	1.2 g
NaCl	4.4 g	11 g	13.2 g
K ₂ HPO ₄ soln	2 ml	5 ml	6 ml
FeSO ₄ soln	1 ml	2.5 ml	3 ml
Trace Minerals (1,000X)	0.2 ml	0.5 ml	0.6 ml
Vitamin soln (100X)	2 ml	5 ml	6 ml
Resazurin soln	0.2 ml	0.5 ml	0.6 ml
NaAcetate·3H ₂ O	0.28 g	0.7 g	0.84 g
Casamino acids (McCas only)	0.4 g	1 g	1.2 g
Difco Noble agar	3 g	7.5 g	9 g

****Add just before autoclaving:****

dithiothreitol	0.1 g	0.25 g	0.3 g
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****Have ready but do not add yet (McCas only):****

cysteine·H ₂ O·HCl	0.1 g	0.25 g	0.3 g
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(1) Immediately before autoclaving, add the dithiothreitol and swirl.

(2) Autoclave

(3) Take out of autoclave and begin gassing with N₂CO₂. While still hot, add cysteine·H₂O·HCl and swirl (**McCas only**).

(4) Place in hot water bath and let cool under stream of N₂CO₂.

(5) Bring into anaerobic chamber and pour plates.

- For plates with antibiotics, add antibiotics from sterile anaerobic stocks before pouring plates.
- For analog plates, before pouring plates, add analog slowly with a syringe while swirling medium to ensure that analog does not precipitate.

