HIGG medium

References

Original recipe:
Cohen-Bazire, G., Sistrom, W. R., Stanier, R. Y. 1957. Kinetic studies of pigment by non-sulfur purple bacteria. J. Cell Comp. Physiol. 49:25-68.

Modified for *Caulobacter*:
Poindexter, J. S. 1978. Selection for nonbuoyant morphological mutants of *Caulobacter crescentus*. J. Bact. 135:1141-1145.

Phosphate Buffer:
Stoll, V. S., Blanchard, J. S. 1990. Buffers: Principles and Practice. from *Guide to Protein Purification*. Murray P. Deutscher, ed. Academic Press, Inc. San Diego.

Stock solutions needed

Metals "44" per 100 ml (for addition to Hutner base)

Disodium EDTA	250.0 mg → 1.3 ml 0.5M EDTA
$ZnSO_4 \cdot 7H_2O$	1095.0 mg
$FeSO_4 \cdot 7H_2O$	500.0 mg
$MnSO_4 \cdot H_2O$	154.0 mg
$CuSO_4 \cdot 5H_2O$	39.2 mg
$Co(NO_3)_2 \cdot 6H_2O$	24.8
$Na_2B_4O_7 \cdot 10H_2O$	17.7 mg

A few drops of sulphuric acid are added to retard precipitation.

Concentrated Hutner Base (100%)
(referred to in Poindextor paper as "double strength")
(* omit vitamins if using for *Caulobacter*):

Nitrilotriacetic acid	10.0 g
$MgSO_4$	14.45 g → 29.59 g heptahydrate
$CaCl_2 \cdot 2H_2O$	3.335 g → 2.518 g anhydrous
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	9.25 mg
$FeSO_4 \cdot 7H_2O$	99.0 mg
*Nicotinic acid	50 mg
*Thiamin HCl	25.0 mg
*Biotin	0.5 mg
Metals "44"	50.0 ml
Distilled H_2O to 1000 ml	
Sterile filter (do not autoclave)	

When making concentrated base, first disolve the nitrilotriacetic acid and neutralize with KOH (it takes approximately 10 ml of 10N solution). ~ 5.6 g KOH ← Add the rest of the ingredients and adjust pH to 6.6-6.8 before adjusting to volume. This is very important or your Fe will precipitate out. (You will see some precipitation as you adjust the pH but things should go back in solution).

Na_2HPO_4-KH_2PO_4 buffer, pH 7.0

0.5 M Phosphate Buffer, pH= 7.0
Be careful to use the correct amount of chemical as these come in multiple forms (anhydrous or hydrated) Calculate the amount of dry chemical you will need to make 0.5 M solutions from the molecular weight of the form of phosphate which you have to use)

-61 ml 0.5 M Na_2HPO_4
-39 ml 0.5 M KH_2PO_4
The pH should be 7.0 upon adding these two components together to make 100 ml; Autoclave.

1 M imidazole pH=7.0 (adjusted with HCl)

20 % glucose (filter sterilized)

20 % sodium glutamate at pH=7.0 (filter sterilized)

1 M NH_4Cl, autoclaved

HIGG medium (1 liter):

The most convenient way to make HIGG is to autoclave the required amount of water, let it cool, and then add each component.

Stock	Volume added for 100 ml	Volume added for 250 ml	Volume added for 500 ml	Volume added for 1 liter	Final concentration
1M imidazole, pH=7	0.5 ml	1.25 ml	2.5 ml	5 ml	5 mM
Hutner concentrated base (100 %)	2 ml	5 ml	10 ml	20 ml	2 %
50mM CaCl2	2 ml	5 ml	10 ml	20ml	1mM
20% glucose	0.75 ml	1.88 ml	3.75 ml	7.5 ml	0.15 %
Nitrogen sources (use both)					
20 % sodium glutamate	0.75 ml	1.88 ml	3.75 ml	7.5 ml	0.15 % (8.9 mM)
1 M NH_4Cl	0.89 ml	2.23 ml	4.45 ml	8.9 ml	8.9 mM
Phosphate (500 mM)	6 µL	15 µL	30 µL	60 µL	30 µM (low)
(choose one)	40 µL	100 µL	200 µL	400 µL	0.2 mM balanced P and C
	200 µL	0.5 ml	1 ml	2 ml	1 mM (high)
	2 ml	5 ml	10 ml	20 ml	10 mM (high)

* the published HIGG recipe uses sodium glutamate as a nitrogen source; however, according to Jeanne Poindextor the recipe should include equimolar amounts of both sodium glutamate and NH_4Cl

Doubling time when phosphate is in excess should be approx. 2.5 hours.

Phosphate becomes growth yield limiting at 100 µM and growth rate limiting at 20 µM in the presence of 0.2 % glucose. 1 mM phosphate is excess phosphate (non-limiting) and 10 mM can also be used.

When cells are limited for phosphate with respect to carbon, they accumulate poly-ß-hydroxybutiric acid (PHB) granules; when cells are limited for carbon with respect to phosphate, they accumulate polyphosphate. Simultaneous exhaustion of phosphate and carbon result in cells lacking either storage polymer [this occurs at 0.2 mM phosphate and 0.2 mM (glucose + glutamate)].