

1. Media Preparation

1a. Nitrate Mineral Salt (NMS) media preparation (Whittenbury et al., 1970)

- Add following ingredients in 700mL of water and dissolve before make-up to 1L.

Ingredient	Amount (per liter)
MgSO ₄ •7H ₂ O	1.0 g
KNO ₃	1.0 g
CaCl ₂ •H ₂ O	0.2 g
3.8% (w/v) solution Fe-EDTA	0.1 ml
0.1% (w/v) NaMo•4H ₂ O	0.5 ml
Trace element solution (recipe below)	1.0 ml

NOTE: The Fe-EDTA and NaMo₄•4(H₂O)₄ solutions are typically made at 1X and stored at 4°C; Fe-EDTA solution not to be exposed to light to prevent photo-degradation.

- If you want to prepare NMS-agar plates, add 18g Agar for 1 L of NMS media (add magnetic stir bar, may help to stir the media while adding below components)
- Autoclave the medium and then add following ingredients.

Ingredient	Amount (per liter)
Phosphate stock solution (1X) (recipe below)	10mL
Vitamin Stock solution (10X) (recipe below)	1mL

- Copper can be added as required. CuCl₂ (10 mM) is usually prepared, filter sterilized and stored for use.
- NMS-liquid media (NMS-liquid) use 1 μM-final Cu concentration. For 1 μM final Cu-concentration 10 μL of 10 mM CuCl₂ is mixed with 100 mL of NMS in culture flask (DF=10,000x).
- NMS-solid media (NMS-agar) contain 10 μM-final Cu concentration. For 10 μM final Cu-concentration 100 μL of 10 mM CuCl₂ is mixed with 100 mL of NMS in culture flask (DF=1,000x).

Stock Trace element solution (1x)

- Add below components to 700 mL water and dissolve. Then add additional water to 1 L.

Ingredient	Amount (per liter)
FeSO ₄ •7H ₂ O	500 mg
ZnSO ₄ •7H ₂ O	400 mg
MnCl ₂ •7H ₂ O	20 mg
CoCl ₂ •6H ₂ O	50 mg
NiCl ₂ •6H ₂ O	10 mg
H ₃ BO ₃ (boric acid)	15 mg
EDTA	250 mg

NOTE: This solution need not to be sterilized as it is added to the base components of NMS medium prior to autoclaving. Stock solutions of 1X or 10X can be prepared and stored at 4°C

Stock Phosphate solution (1x)

- Add above components to 700 mL water and dissolve. Then add additional water to 1 L. Autoclave and store at room temperature.

Ingredient	Amount (per liter)
KH ₂ PO ₄	26 g
Na ₂ HPO ₄ •7(H ₂ O) (or Na ₂ HPO ₄)	62 g (or 33g)

NOTE: pH should be 6.8

Stock Vitamin solution (10x)

- Add above components to 700 mls water and dissolve. Then add additional water to 1 liter and filter sterilize using a 0.2 μ m filter.

Ingredient	Amount (per liter)
Biotin	20 mg
Folic acid	20 mg
Thiamine HCl	50 mg
Ca pantothenate	50 mg
Vitamin B12	1 mg
Riboflavin	50 mg
Nicotiamide	50 mg

NOTE: It is easier to make 10X stock solutions and then dilute to 1X prior to use. Store at 4°C. It is important that the vitamin solution not to be exposed to light to prevent photo-degradation

1b. NMS-Antibiotic Plates/media preparation

- Some of the OB3b-mutants required specific antibiotics to retain the plasmids. Hence, it's necessary to maintain the cultures in appropriate plates or enriched in NMS-liquid supplied with antibiotics.
- Follow the NMS-liquid or agar media preparation protocol as detailed above (i.e., 1a). Add antibiotics when the media cool down to < 60°C.
- Prepare the antibiotic solutions, filter sterilize and store at appropriate temperature.
- Add to NMS-liquid or agar as appropriate (check below table for reference)

Ingredient (Storage requirements)	Stock Concentration (mg/mL)	Amount (µL) of stock solution for 1L of NMS	Final concentration in NMS (µg/mL)
Gentamicin (15-30°C)	50	50	2.5
Ampicillin (-20°C)	100	100	100
Kanamycin (4°C)	50	200	10
Spectinomycin (-20°C)	100	200	20

NOTE: The antibiotics stocks cannot be stored for more than one year.