

1630e: Synthetic Crenarchaeota Medium (SCM)

This recipe contains strain-specific modifications for *Nitrosopumilus zostrerae* DSM 116344 *

Final pH: 7.6

Final volume: 1000 ml

Basic salt water medium

NaCl	26.00	g
MgSO ₄ x 7 H ₂ O	5.00	g
MgCl ₂ x 6 H ₂ O	5.00	g
CaCl ₂ x 2 H ₂ O	1.50	g
KBr	0.10	g
NaHCO₃ buffer (1M)	2.00	ml
HEPES buffer solution	10.00	ml
KH₂PO₄ solution (2.93 mM)	5.00	ml
Trace element solution (Nitrososphaera)	1.00	ml
FeNaEDTA solution (7.5 mM)	1.00	ml
NH₄Cl (1 M)	2.00	ml
Double distilled water	1000.00	ml
alpha-ketoglutarate solution (20mM)	5.00	ml

1. Incubate all used glass ware and magnetic stirring rods in 0.1 M HCl and rinse 3x with double distilled water to remove residual detergents (always check visual for soap bubbles).
2. Autoclave the basic salt water medium and cool to room temperature. This medium should be free of any precipitations!
3. Aseptically, add per L of basic salt water medium the following solutions from sterile stocks (do not store complemented SCM): Trace element solution (Nitrososphaera), FeNaEDTA solution, NaHCO₃ solution, HEPES buffer solution, NH₄Cl solution, KH₂PO₄ solution
4. Adjust pH to 7.6.

* add from sterile filtered stock solution 5 mL/L alpha-ketoglutarate (20 mM)

HEPES buffer solution (from medium 1630)

NaOH (pellets)	24.00	g
HEPES (free acid)	238.40	g
Distilled water	1000.00	ml

First dissolve NaOH in ca 600 ml water, then add HEPES, adjust pH to 7.5 and fill up to 1000 ml volume.

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Trace element solution (Nitrososphaera) (from medium 1630c)

HCl (12.5M)	8.00	mL
H ₃ BO ₃	30.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
ZnSO ₄ x 7 H ₂ O	144.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Double distilled water	1000.00	ml

This solution contains no iron! Sterilise by autoclaving or filtration (0.2 µm) and store in the dark at 4 °C.

FeNaEDTA solution (7.5 mM) (from medium 1630c)

FeNa-EDTA	2753.00	mg
Double distilled water	1000.00	ml

Sterilise by filtration (0.2 µm) and store in the dark at 4 °C.

NaHCO₃ buffer (1M) (from medium 1630c)

NaHCO ₃	4.20	g
Double distilled water	50.00	ml

Dissolve 4.2 g of NaHCO₃ in 50 mL Double distilled water (use a small beaker with stirring rod to dissolve) and sterilize by filtration (0.2 µm) in the laminar flow hood. Store in a sterile 50 mL falcon tube at 4 °C. Avoid high headspace to liquid ratios to ensure stable concentration.

NH₄Cl (1 M) (from medium 1630c)

NH ₄ Cl	2.67	g
Double distilled water	50.00	ml

1. Dissolve 2.67 g ammonium chloride in 50 mL Double distilled water.
2. Filtrate (0.2 µm).

KH₂PO₄ solution (2.93 mM)

KH ₂ PO ₄	0.40	g
Double distilled water	1000.00	ml



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alpha-ketoglutarate solution (20mM)

alpha-ketoglutarate	20.00	mM
Double distilled water	1000.00	ml