

## MN MEDIUM

### PROTOCOL

- Add solutions according to the volume of sea water (ideally filtered before by GF/C: grade 1.2  $\mu\text{m}$ ) and then adjust to the final volume with distilled/deionized water before autoclaving.
- After autoclaving and cooling, add vitamin B12 (final concentration in the medium 10 $\mu\text{g/L}$ ). Defrost stock solution (1000x concentrated) and in the flow chamber (aseptic conditions), add the vitamin to the medium through filtration-sterilization (0.2  $\mu\text{m}$ ).
- To prepare solid medium, previously add 10-15g agar per 1L of medium (1- 1.5% w/v). In this case, add vitamin B12 when the temperature of the medium is close to 55-60 $^{\circ}\text{C}$ , immediately before of distribute the medium by plates (in aseptic conditions and after gently shaking to mix the vitamin).

Reagent	Name	ml/L
NaNO <sub>3</sub>	Sodium Nitrate	5
K <sub>2</sub> HPO <sub>4</sub> ·3H <sub>2</sub> O	Potassium Phosphate Dibasic Trihydrate	0.5
MgSO <sub>4</sub> ·7H <sub>2</sub> O	Magnesium Sulfate Heptahydrate	0.5
CaCl <sub>2</sub> ·2H <sub>2</sub> O	Calcium Chloride Dihydrate	0.5
C <sub>6</sub> H <sub>8</sub> O	Citric Acid	0.5
C <sub>6</sub> H <sub>5</sub> +4yFexNyO7	Ammonium Ferric Citrate	0.5
C <sub>10</sub> H <sub>16</sub> N <sub>2</sub> O <sub>8</sub>	EDTA	0.5
Na <sub>2</sub> CO <sub>3</sub>	Sodium Carbonate	1
-	Trace metal Stock solution A5+Co	1
Sea Water	-	750
dH <sub>2</sub> O	Deionized Water	1L

### Composition of stock Solutions:

#### Trace metal Stock solution A5+Co

Reagent	Name	g/L
H <sub>3</sub> BO <sub>3</sub>	Boric acid	2.86
MnCl <sub>2</sub> ·4H <sub>2</sub> O	Manganese dichloride tetrahydrate	1.81
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	Zinc Sulphate Heptahydrate	0.222
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	-	0.390
CuSO <sub>4</sub> ·5H <sub>2</sub> O	Copper sulfate pentahydrate	0.079
Co(NO <sub>3</sub> ) <sub>2</sub> ·6H <sub>2</sub> O	Cobalt(II) nitrate hexahydrate	0.049