

441: DIAZOTROPHIC MEDIUM (RBA)

This recipe contains strain-specific modifications for *Azorhizophilus paspali* DSM 388 *

Final volume: 1008 ml

Solution A	953.00	ml
Solution B	50.00	ml
Standard vitamin solution	5.00	ml

1. Sterilize solution A separately at 121°C for 15 min., cool to 50°C and then mix aseptically with filter-sterilized solution B and 5.0 ml of filter-sterilized standard vitamin solution (see medium 428).
2. RBA is an ammonium-free medium which has successfully been used for the isolation, growth and purity check of a broad spectrum of nitrogen fixing bacteria (Ref. 3363). For microaerophilic nitrogen-fixing bacteria use semisolid medium with 0.3% end concentration of agar and incubate the liquid cultures under 10% (v/v) air and 90% (v/v) N₂.

* Only liquid

Solution A

KH ₂ PO ₄	0.10	g
K ₂ HPO ₄	0.90	g
NaCl	0.10	g
CaCl ₂ x 2 H ₂ O	0.10	g
MgSO ₄ x 7 H ₂ O	0.10	g
Na ₂ MoO ₄ x 2 H ₂ O	5.00	mg
NaVO ₃ x H ₂ O	5.00	mg
MnSO ₄ x H ₂ O	5.00	mg
FeSO ₄ x 7 H ₂ O	0.01	g
Yeast extract	0.05	g
Trace element solution SL-6	3.00	ml
Distilled water	950.00	ml

~~Agar, if required 15.00 g~~

Adjust pH to 7.3.

Solution B

Disodium succinate	1.00	g
DL-Malate	2.00	g
Na-pyruvate	1.00	g
D-Mannitol	2.00	g

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D-Glucose	2.00	g
Distilled water	50.00	ml

Adjust pH to 7.3.

Trace element solution SL-6 (from medium 27)

ZnSO ₄ x 7 H ₂ O	0.10	g
MnCl ₂ x 4 H ₂ O	0.03	g
H ₃ BO ₃	0.30	g
CoCl ₂ x 6 H ₂ O	0.20	g
CuCl ₂ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.02	g
Na ₂ MoO ₄ x 2 H ₂ O	0.03	g
Distilled water	1000.00	ml

Standard vitamin solution (from medium 428)

Riboflavin	10.00	mg
Thiamine-HCl x 2 H ₂ O	50.00	mg
Nicotinic acid	50.00	mg
Pyridoxine hydrochloride	50.00	mg
Calcium pantothenate	50.00	mg
Biotin	0.10	mg
Folic acid	0.20	mg
Vitamin B ₁₂	1.00	mg
Distilled water	100.00	ml