

465d: MINERAL MEDIUM WITH BENZYL CYANIDE AS NITROGEN SOURCE

This recipe contains strain-specific modifications for *Rhodococcus erythropolis* DSM 9675 *

Final pH: 7.2

Final volume: 1000 ml

Main sol. 465 (modified)	1000.00	ml
Glucose	1.80	g/l
Benzylcyanide	120.00	mg/l
Sodium succinate	1.60	g/l

Prepare and autoclave medium 465 without ammonium sulfate. Add 120 mg/l benzylcyanide (not sterilized) to the incubation atmosphere or directly to the medium.

* Instead of 1.8 g/l glucose add 1.6 g/l sodium succinate from a sterile solution.

Main sol. 465 (modified)

Na ₂ HPO ₄ x 2 H ₂ O	3.50	g
KH ₂ PO ₄	1.00	g
MgCl ₂ x 6 H ₂ O	0.10	g
Ca(NO ₃) ₂ x 4 H ₂ O	0.05	g
Trace element solution SL-4	1.00	ml
Distilled water	1000.00	ml

1. pH 7.25

2. Rehydrate and cultivate lyophilized cells in the complex medium recommended for the specific strain (e.g. medium 1, 220, 535 or 830). After this reactivation, cultivate on mineral medium with the appropriate carbon source.

Trace element solution SL-4 (from medium 14)

Na ₂ -EDTA	0.50	g
FeSO ₄ x 7 H ₂ O	0.20	g
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

First dissolve EDTA in distilled water and adjust pH to 7.0 using 2 N NaOH; then add other

compounds.