

645. MIDDLEBROOK MEDIUM

Bacto Middlebrook 7H10 agar (Difco 262710)	19.000	g
Glycerol	5.000	ml
Distilled water	900.000	ml

Sterilize for 15 min by 121°C cool to 50 - 55°C. Add 100 ml Middlebrook OADC enrichment (Becton Dickinson 212240) under aseptic conditions and mix thoroughly.

If you can't order the commercial media then prepare the media in this way:

Bacto Middlebrook 7H10 agar:

(NH ₄) ₂ SO ₄	0.500	g
KH ₂ PO ₄	1.500	g
Na ₂ HPO ₄	1.500	g
Na-citrate	0.400	g
MgSO ₄	0.025	g
CaCl ₂	0.500	mg
ZnSO ₄	1.000	mg
CuSO ₄	1.000	mg
L-Glutamic acid	0.500	g
Ferric ammonium citrate	0.040	g
Pyridoxine	1.000	mg
Biotin	0.500	mg
Malachite green	0.250	mg
Bacto agar	15.000	g
Distilled water	1000.000	ml

Final pH at 25°C 6.6 ± 0.2.

Bacto Middlebrook OADC enrichment:

Oleic acid	0.050	g
Albumin fraction V, bovine	5.000	g
Dextrose	2.000	g
Catalase (beef)	4.000	mg
NaCl	0.850	g
Distilled water	100.000	ml