

## Main sol. 1147

EDTA	10.00	mg
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	200.00	mg
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	75.00	mg
NaCl	15.00	g
NH <sub>4</sub> Cl	0.52	g
DL-Malic acid	4.00	g
K <sub>2</sub> HPO <sub>4</sub>	1.00	g
BICINE buffer (Sigma)	10.00	mM
<b>Trace elements</b>	1.00	ml
Distilled water	1000.00	ml

### 1. Filter-sterilize and add after autoclaving:

Thiamine	1.00	mg
Vitamin B <sub>12</sub>	20.00	µg
Biotin	30.00	µg

2. The pH of the medium was adjusted to 9.5 by addition of NaOH and then sterilized by autoclaving. Before inoculating medium RV5, 0.1% (w/v) NaHCO<sub>3</sub> was added to tubes, bottles, or molten media from a 5% (w/v) filter-sterilized stock solution. For routine culture, medium RV5 supplemented with 0.05% (w/v) Difco yeast extract was used.