Main sol. 683

NaCl	5.80	g
$MgCl_2$ x 6 H_2O	0.40	g
KCl	0.30	g
$CaCl_2$ x 2 H_2O	0.15	g
NH_4Cl	0.27	g
KH_2PO_4	0.20	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract (OXOID)	10.00	g
Trypticase peptone (BD BBL)	0.50	g
L-Serine	2.00	g
L-Threonine	2.00	g
N-methylhydantoin (SIGMA)	5.64	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.50	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, cysteine and vitamins) and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Adjust pH to 8.3 with 10 N NaOH, add bicarbonate and cysteine and dissolve. Switch gas phase to 80% N_2 and 20% CO_2 gas mixture and flush only the head space of the medium vessel. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave 15 min at 121°C. Add vitamins to the autoclaved medium from an anoxic stock solution prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 8.3, if necessary.

2. Note: If creatinine is used as the substrate replacing N-methylhydantoin, prepare separately an anoxic, filter sterilized stock solution of creatinine under 100% N_2 gas atmosphere.