ATCC medium: 2453 DH medium

Complete medium:
Medium D (20X) (see below)..... 50.0 ml
HEPES.....1.2 g
Distilled deionized water.....1.0 L

Adjust pH to 8.24-8.26 then autoclave at 121C.

Leave to cool in dark cupboard overnight. The next day test the pH to make sure it is appropriate. When first prepared and autoclaved, Medium D may be slightly cloudy or turbid. It should clear as it cools and remain clear in storage. The medium should be stored in the dark for 1-2 days before using for culture work.

Medium D--20X Concentration:
NTA(nitrilotriacetic acid).....2.0 g
Micronutrient solution (see below).....10.0 ml
$FeCl_3$ solution (0.29 g/liter) .....20.0 ml
$CaSO_4 \cdot 2H_2O$ .....1.2 g
$MgSO_4 \cdot 7H_2O$ .....2.0 g
NaCl.....0.16 g
KNO_3 .....2.0 g
$NaNO_3$ .....14.0 g
Na_2HPO_4 .....2.2 g
Distilled deionized water.....1.0 L

Filter-sterilize to store for longer than one week.

Micronutrient Solution:
H_2SO_4 (concentrated) .....0.5 ml
$MnSO_4 \cdot H_2O$ .....2.28 g
$ZnSO_4 \cdot 7H_2O$ .....0.50 g
H_3BO_3 .....0.50 g
$CuSO_4 \cdot 5H_2O$ .....0.025 g
$Na_2MoO_4 \cdot 2H_2O$.....0.025 g
$CoCl_2 \cdot 6H_2O$ .....0.045 g
Distilled deionized water.....1.0 L