3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

51295 Haemophilus Medium

Haemophilus Medium was developed from Sigma-Aldrich for optimized growth of *Haemophilus* species. Cultures of *H. influenzae* reached an OD at 640 of greater than or equal to 4.5 within 24 hours.

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Appearance:	Light beige coloured, homogeneous, free flowing powder.
Colour and Clarity:	Dark yellow coloured, clear solution.

Directions:
Suspend 81.7 g in 1 litre distilled water. Bring to the boil to dissolve. Sterilize by autoclaving at 121°C for 15 minutes. Haemophilus medium must be supplemented with 44 ml/l glycerol (Cat. No. 49767), 10 mg/l β-NAD (Cat. No. 43410) and 30 mg/l hemin (Cat. No. 51280) for complete medium.

Preparation of Working Stocks:
Cells from one colony of *Haemophilus* species were transferred to 50 ml of sterile Haemophilus Medium in a 250 ml baffled shake flask (Catalog No. F-0644) with a silicone sponge closure (Catalog No. C-0921). The flask was shaken overnight at 300 rpm (New Brunswick G-2 rotary shaker) at 37 °C. The cells were then centrifuged at 800 x g for at least 10 minutes and the pellet was resuspended in 20 ml fresh Haemophilis Broth. This working stock can be used immediately or aliquoted into cryogenic freezing vials and stored at -70°C.

Suitability Assay:
Starter culture was prepared by adding 0.3 ml working stock to 50 ml of sterile Haemophilus Medium and shaken overnight at 300 rpm at 37°C as in the preparation of the working solution above. 1 ml of this starter culture was immediately added to 50 ml of fresh Haemophilus Medium in a baffled shake flask. The cells were shaken at 37°C as above and the OD 640 was measured at various time points for a 24 hour period. The OD at 640 was 4.5 or greater within 24 hours.
NOTE: During log phase of growth *H. influenzae* forms cocci. As a result, the relationship between the number of cells and the OD 640 will change during this phase.

1. Hemin stock is prepared by suspending Hemin (Cat. No. 51280) to a final concentration of 10 mg/ml in 1.4 M ammonium hydroxide.
2. β-NAD stock is prepared by disolving β-NAD (Cat. No. 43410) in distilled or deionized water to a final concentration of 10 mg/ml. The solution is sterile filtered and used immediatly or stored in sterile cryogenic freezing vials at -20°C.