Colonization Medium w/ Dulbeccos Phosphate Buffer

M1239

Colonization Medium is used to prepare the solution of enterotoxigenic *Escherichia coli* used for colonization test in HeLa cell lines.

Composition**

Ingredients	Gms / Litre
Brain heart infusion powder	0.700
Bile salts mixture	0.140
Mannose	1.400
Potassium chloride	0.200
Monopotassium phosphate	0.200
Sodium chloride	8.000
Dipotassium phosphate	1.150
Final pH (at 25°C)	7.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 11.79 grams in 1000 ml distilled water. Mix well and sterilize by filtration through a 0.45 mm membrane. DO NOT AUTOCLAVE OR HEAT the medium.

Principle And Interpretation

This medium is formulated as recommended by APHA (1) for preparing suspension of enterotoxigenic *Escherichia coli* used for colonization test in HeLa cell lines.

Virulence prerequisites for enterotoxigenic strains of *E. coli* include the ability to attach to the jejunal lining to proliferate *in-situ* , and to elaborate one or more toxins. Host specificity is manifested by possession of unique colonization factors like antigens and lectins. Because of commercial non-availability of these factors several types of mammalian cells have been proposed to show colonization, and HeLa is one of them. It was found that many *E.coli* strains attach to HeLa cells with two different attachment patterns i.e. diffused adherence and localized adherence. By adding mannose to the culture medium it was possible to distinguish between mannose-sensitive and mannose-resistant adherence. Mannose-resistant adherence was not related to colonization factor antigens.

Colonization Medium w/ Dulbeccos Phosphate Buffer is used in the HeLa adherence assay.

Brain heart infusion in the medium provides necessary nutrients like amino acids, growth factors and trace ingredients for the growth of enterotoxigenic E. coli. Mannose acts as an energy sources as well as differentiates mannose-sensitive and mannose-resistant adherence. Phosphates buffer the medium. Bile salts mixture inhibits the contaminating flora while sodium chloride maintains the osmotic equilibrium.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light to medium amber coloured, clear solution without any precipitate

Reaction
Reaction of 1.18% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

Cultural Response
M1239: Satisfactory results are obtained when used for preparation of suspension of enterotoxigenic Escherichia coli used for HeLa cells test for colonisation .

Storage and Shelf Life

Store dehydrated powder and the prepared medium at 2-8º C in tightly closed container. Use before expiry date on the label

Reference

1. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.

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