LB Agar (Lennox)

LAB 174

Description
This is a nutritionally rich medium containing half the sodium chloride level of LB agar (LAB168). This allows the researcher to select the optimum salt concentration for his experiment. This medium can also be used for plasmid replication experiments. Nutritionally rich media are required for molecular biology applications as the strains used are often derived from *Escherichia coli* K12, which is deficient in B vitamin production.

Formula	g/litre
Tryptone	10.0
Yeast Extract	5.0
Sodium chloride	5.0
Agar	15.0

Method for reconstitution
Weigh 35.0 grams of powder and disperse in 1 litre of deionised water. Allow the mixture to soak for 10 minutes, swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add filter sterilised antibiotic as required. Pour into sterile Petri dishes and allow the medium to set. Dry the surface prior to inoculation.

Addition of Substrate

Prepare the X-Gal solution by dissolving in DMF, to give a concentration of 20mg/ml. Once dissolved, spread 40µl as a surface layer over the top of the agar and allow to dry. Also spread 4µl of a solution of IPTG (200mg/ml).

Appearance: Straw, clear gel.

pH: 7.0 ± 0.2

Minimum QC organisms - βgal reaction:

Escherichia coli DH5a (ATCC® 53868)
Lac Z+ve (black if CHE-gal is present in the medium)
Escherichia coli DH5a, Lac Z -ve (remains cream even in the present of CHE-gal)

Storage of Prepared Medium: Plates – up to 7 days at 2-8°C in the dark.

Inoculation: Surface; either spread over entire surface for colony count or streaking for single colonies.

Incubation: 37°C aerobically for 16-18 hours.

Interpretation: Using the base medium alone, all colonies will appear cream. Alternatively, if a chromogen is included, examine for the presence of cream colonies, which indicates a successful insertion of the target DNA.

References
Lennox, E.S. (1955). Transduction of linked genetic characters of the host by bacteriophage P1. Virology 1, 190.

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Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Laboratory. Cold Spring Harbour New, York.