LB Agar

LAB 168

Description
A nutritious medium designed for rapid bacterial growth, typically sed in molecular biology procedures e.g. in the detection of phage or plasmid transformed bacteria and the maintenance of recombinant strains. This agar contains the required concentration of sodiumchloride to promote replication of plasmids.

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

Method for reconstitution
Weigh 40.0 grams of powder and disperse in 1 litre of deionised water. Swirl to mix and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47°C and add filter sterilised antibiotic if 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). Alternatively, use Harlequin™ LB agar complete (HAL004), which already contains the enzyme substrate and inducer. This eliminates the potentially hazardous use of DMF and prevents variation in the colour of ß-complemented colonies due to differences in substrate concentration.

Appearance: Straw, clear gel.

pH: 7.0 ± 0.2

Minimum QC organisms - β-gal 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 presence of CHE-gal)

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

Inoculation: Typically surface spread over plate to detect cream colonies indicating disruption of ß-complementation. Alternatively, spread for single colonies if required.

Incubation: 37°C aerobically, for 16-18 hours. If a chromogenic substrate is used, the colour of the colonies will substantially increase with prolonged incubation (up to 24 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
Miller, J.H. (1972). Experiments in Molecular Genetics. Cold Spring Harbour Laboratory. Cold Spring Harbour New ,York.

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.