



## **LB Broth**

### **LAB 169**

#### **Description**

A nutrient broth primarily used for the growth and maintenance of *Escherichia coli*. Used as the primary propagation step for donor or recipient cells, when further work is to be performed on LB Agar. This broth contains a high level of sodium chloride to aid the maintenance of plasmids. If working with temperate bacteriophages, such as lambda, the addition of magnesium sulphate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ) at 2 grams per litre is recommended to promote phage absorption.

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

#### **Method for reconstitution**

Weigh 25.0 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.

**Appearance:** Straw, clear liquid.

**pH:**  $7.0 \pm 0.2$

Minimum QC organisms: *Escherichia coli* DH5 (ATCC® 53868)

**Storage of Prepared Medium:** Capped containers – up to 3 months at 15-20°C in the dark.

**Inoculation:** As per normal techniques, using a pure culture of donor/recipient cells.

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

**Interpretation:** Examine all tubes for turbidity, indicating growth.

#### **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.