

The Standard Plate Incorporation Assay

Assay Setup

Assay Performance

Collect Results

Day 0

Day 1

Day 2

Day 3

Label Minimal Glucose Agar Plates and sterile test tubes (13 x 100mm) with test chemical doses

Inoculate cultures* (15 – 18 h prior to assay performance)

Arrange sterile test tubes in heat block ($\approx 45^{\circ}\text{C}$)

Melt supplemented top agar; maintain at $45^{\circ}\text{C} - 48^{\circ}\text{C}$

Grow cultures to $1 - 2 \times 10^9$ cfu/ml*

Prepare chemical dilutions (10x desired dose)

Prepare S9 Mix; keep on ice

To each test tube add:

2 ml top agar

+

100 μl chemical dose

One at a time, to each test tube add:

50 μl S9 Mix or buffer

+

100 μl strain culture

immediately

Vortex mix tube, pour onto MGA plate (covering evenly)

Allow top agar to harden; Invert

Incubate at 37°C for 48 h

Repeat for each test tube

Count colonies (# of revertant colonies/plate)