

The Preincubation 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

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

Prepare chemical dilutions (10x desired dose)

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

Prepare S9 Mix; keep on ice

Arrange sterile test tubes in a rack (room temperature)

Melt supplemented top agar; maintain at 45°C - 48°C

To each test tube add:

50 µl S9 Mix or buffer

+

100 µl chemical dose

+

100 µl strain culture

Rotate rack gently to mix tubes

Incubate at 37°C (with shaking) for 20 - 30 min

One at a time, to each test tube add:

2 ml top agar

immediately

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

Repeat for each test tube

Allow top agar to harden; invert

Incubate at 37°C for 48 h

Count colonies (# of revertant colonies/plate)

*Reference MOLTOX[®] Technical Bulletin *Lyophilized Culture Discs*