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Growing Broth Cultures using Moltox™

ST– and EC- Discs™

This guidance is for growing Moltox's lyophilized *S. typhimurium* and *E. coli* bacterial discs for use in the bacterial reverse mutation assay (i.e., Ames Assay).

General Information on growing these strains-

Nutrients

Oxoid Nutrient Broth No. 2 is **required** to grow these bacterial strains. Other brands of nutrient broth do not perform as well and may actually be detrimental to the culture resulting in inaccurate results.

Oxygen

Grow these organisms aerobically. Use a vessel that is 3—5x the volume of broth. Ensure adequate aeration by leaving the vessel cap or plug loose and orienting your vessel to achieve maximum broth surface area (i.e., slant tubes if using).

Incubation Conditions

Grow these cultures in a dry incubator at 35 ± 2°C.
Shaking the culture increases aeration and decreases overall incubation time.
Grow these cultures to a density of 1—2 x 10E^9 cfu/ml. **Generally**, this is equal to an OD_{650nm} = 1.0 -1.4. Ideally this would be confirmed with a growth curve study in the user's laboratory. Density can be confirmed through serial dilution and plating.
These cultures are used in the active growing phase. Use them **as they enter** the above density.

Directions for Use:

On the evening prior to use in testing, warm the product vial to room temperature[1]; using sterile forceps or loop, aseptically transfer a disc to 25—30 mls Oxoid Nutrient Broth #2 . Hold the culture **stationary** at 37°C overnight[2]. Early the next morning incubate with shaking at 37°C until a density of 1–2 × 10^9 bacteria/mL is achieved, at which point the culture will be virtually opaque. Under optimal conditions this bacteria may double in number approximately every 30 minutes or less. Do not overgrow the cultures[3].

[1] This prevents condensation from forming in the vial which could impair the performance of the discs.

[2] The number of viable cells in the discs is high enough that, if the culture is shaken overnight, the culture will overgrow and be out of the active growing phase.

[3] If the culture is overgrown, **do not** dilute such culture to the recommended range. Instead, remove a portion of the culture, add to fresh Oxoid Nutrient Broth No. 2 and incubate with shaking , monitoring until the culture enters the recommended density.