MOLTOX AMES ASSAY *STDisc*

APPLICATION AND QUALITY CONTROL STATEMENT

LOT NO.: 5241D **STRAIN:** TA1535 **PREPARATION DATE: February 08, 2018**

PART NO.: 71-1535L **SPECIES:** *S. typhimurium* **EXPIRATION DATE: February 08, 2020**

STORE 2 - 8°C. WARM TO ROOM TEMPERATURE BEFORE OPENING.

ETIOLOGIC AGENT - USE ASEPTIC TECHNIQUE.

NOTE: Disc cultures were prepared from master cultures obtained from Dr. B.N. Ames using a modification of the methods described by the American Type Culture Collection. The bacteria have been fully characterized for appropriate phenotypic characteristics and response to diagnostic mutagens as recommended by OECD guideline 471. The bacteria are not intended for applications in *in vitro* diagnostic procedures. ***For investigational use only by microbiologists trained in the safe handling of bacteria.***

PROCEDURE: On the evening prior to use in testing, warm the product vial to room temperature; using sterile forceps or loop, aseptically transfer a disc to Oxoid #2 nutrient broth. Hold the culture stationary at 37°C overnight. Early the next morning incubate with shaking at 37°C until a density of 1–2 × 10^9 bacteria per mL is achieved, at which point the culture will be virtually opaque - the density can be estimated by direct counting, measurement of optical density or plating dilutions on nutrient agar. Under optimal conditions this bacteria may double in number approximately every 30 minutes or less. Do not overgrow the cultures.

PHENOTYPE CONFIRMATION: A disc from the production lot was cultured in nutrient broth (Oxoid #2) to a target density of 1 – 2 × 10^9 CFU/mL. Using a sterile swab, all sectors of a Moltox Item #21-200 "QUAD-PC" plate were inoculated with the culture. A filter paper disc containing crystal violet was added to Sector II. To detect the absence of excision repair, the culture was streaked on a nutrient agar plate and half the plate was exposed to a 30 W ultraviolet lamp for 8 sec at 33 cm. Both plates were then incubated overnight at 37°C.

Sector	Medium	Diagnostic	Result
I	- L-his	*his*$^-$	no growth
II	+ L-his (CV disc)	*rfa* deep rough	zonal inhibition
III	+ Amp	R-factor plasmid	no growth
IV	+ Amp, +Tet	pAQ1 plasmid	no growth
UV test plate	Nutrient	*uvrA/B*	growth/no growth*

* Unexposed/exposed sides respectively: absence of growth in the UV exposed zone is indicative of excision repair deficiency.

DIAGNOSTIC MUTAGEN RESPONSE: The above culture was also used to determine response characteristics to diagnostic mutagens using triplicate plates as described by Maron & Ames (1983) *Mutation Res.* **113,** 173-215.

Mutagen	Amount/plate	Mean revertants/plate
None	—	6
Daunomycin	6 µg	5
ICR191	1 µg	7
Mitomycin C	0.5 µg	0
NaN_3	1.5 µg	329

VIABILITY: Viability was assessed by re-suspending discs in nutrient broth before dilution in saline and plating on nutrient agar.

An average of 8.5 × 10^5 CFU/disc was observed.

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Approved: Kathy Pedersen 2/16/2018