Genotype Confirmation Testing of E. coli WP2 Ames Strains

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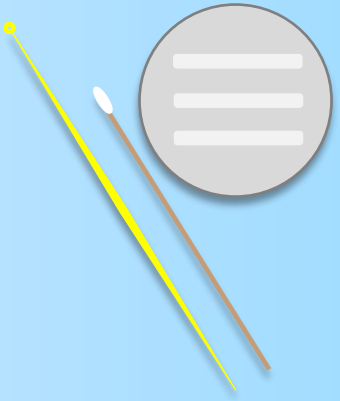
Molecular Toxicology, Inc.

Genotype	Test Media	Assay Setup – Day 0	Assay Performance – Day 1	Expected Results – Day 2
trpE	MGA[1] & MGA[1] w/ 10 - 50 µg/mL tryptophan	Inoculate cultures[2] (15 – 18 h prior to assay performance)	Grow cultures to 1 – 2 x 10^9 cfu/ml[2]; Using a 10 µl inoculating loop[3] or a swab[3, 4], streak culture(s) across MGA(s)	No growth on MGA; growth on MGA w/tryptophan
pKM101[5]	MGA[1] w/ 10 - 50 µg/mL tryptophan & 24 µg/mL ampicillin	Prepare phenotype plates (if applicable)	Incubate *overnight*, 37°C	Growth of pKM101-containing strains only[2]
uvrA[6, 7]	MGA[1] w/ 10 - 50 µg/mL tryptophan[8]			No growth of *uvrA*-containing strains on +UV side[2, 6, 7]



[1] Minimal Glucose Agar. Reference Mortelmans and Zeiger (2000), The Ames *Salmonella*/microsome mutagenicity assay, *Mut Res.* 455, pp 29-70.
[2] Reference MOLTOX® Technical Bulletin *Lyophilized Culture Discs.*
[3] Sterile
[4] Press swab against the side of the growth vessel to remove excess broth prior to inoculating MGA plate. This helps prevent nutrient carryover to the MGA plate which can cause erroneous results.
[5] Streak a pKM101-containing and a pKM101-free strain on the same plate for growth comparison after incubation. Reference MOLTOX® Technical Bulletin *Lyophilized Culture Discs.*
[6] Streak a WP2 and a WP2 *uvrA* culture(s) across plate[2]. With dish lid off, cover ½ the plate and expose the uncovered side to a 15W UV lamp at a distance of 33 cm. Expose non-pKM101 strains for 6 sec, pKM101-containing strains for 8 sec.
[7] Alternate *uvrA* test: After streaking, apply a filter paper disc containing 0.2 µg Mitomycin C to the center of the streak. *uvrA*-containing strains will exhibit a halo around the disc after incubation.
[8] Nutrient Agar prepared with Oxoid Broth No.2 can also be used.