

# Correction Notice: Microbial Mutagenicity Assay: Ames Test

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We checked the composition of Vogel-Bonner media and other media and salts in our publication (<https://doi.org/10.21769/BioProtoc.2763>) and found some mistakes in the units. New, correct units are as follows (in bold):

## Recipes

### 1. Vogel-Bonner medium E (50x)

*For Minimal agar (Recipe 9)*

Ingredients	Per 500 ml
Warm distilled H <sub>2</sub> O (40-50 °C)	335 ml
Magnesium sulfate (MgSO <sub>4</sub> •7H <sub>2</sub> O)	5 g
Citric acid monohydrate	50 g
Potassium phosphate, dibasic (anhydrous) (K <sub>2</sub> HPO <sub>4</sub> )	250 g
Sodium ammonium phosphate (NaNH <sub>4</sub> HPO <sub>4</sub> •4H <sub>2</sub> O)	87.5 g

- Salts are added to the warm water in a flask. Place the flask on a hot plate
- After each salt dissolves entirely, transfer the solution into glass bottles and autoclave for 20 min at 121 °C
- When the solution gets cool, cap the bottle tightly
- Store the solution at 4 °C

### 3. Salt solution (1.65 M KCl + 0.4 M MgCl<sub>2</sub>)

*For S9 hepatic fraction*

Ingredients	Per 250 ml
Potassium chloride (KCl)	30.75 g
Magnesium chloride (MgCl <sub>2</sub> •6H <sub>2</sub> O)	20.35 g
Distilled H <sub>2</sub> O to final concentration of	250 ml

All the components are dissolved in water. The solution is autoclaved for 20 min, at 121 °C and then stored in refrigerator.

### 4. 0.2 M sodium phosphate buffer, pH 7.4

*For S9 hepatic fraction*

Ingredients	Per 250 ml
0.2 M sodium dihydrogen phosphate (NaH <sub>2</sub> PO <sub>4</sub> •H <sub>2</sub> O)	30 ml (6.9 g/250 ml)
0.2 M disodium hydrogen phosphate (Na <sub>2</sub> HPO <sub>4</sub> )	220 ml (7.1 g/250 ml)

Adjust pH to 7.4. Sterilize the buffer by autoclaving for 20 min at 121 °C

### 6. 1 M glucose-6-phosphate

*For S9 hepatic fraction*

Ingredients	Per 5 ml
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|---|--------|
| Glucose-6-phosphate (G-6-P)   | 1.41 g |
| Sterile distilled H <sub>2</sub> O  | 5 ml   |
| Glucose-6-phosphate is dissolved in the 5 ml distilled water and mixed by vortexing. Tubes are placed in an ice bath. The solution can be used for up to six months |        |
7. Ampicillin solution (4 mg/ml)
 

*Used in tests of ampicillin resistance*

Master plates for R-factor strains

Ingredients	Per 500 ml
Ampicillin trihydrate	0.4 g
Sodium hydroxide (0.02 N)	50 ml

Ampicillin trihydrate is dissolved in the 50 ml of NaOH (0.02 N) and mixed by vortexing. Tubes are placed in an ice bath
  8. Crystal violet solution (0.1%)
 

*Used in tests for crystal violet sensitivity (to confirm rfa mutation)*

Ingredients	Per 500 ml
Crystal violet	0.05 g
Distilled H <sub>2</sub> O	50 ml
  9. Minimal glucose plates
 

*Used in Mutagenic bioassay*

Ingredients	Per 500 ml
Agar	7.5 g
Distilled H <sub>2</sub> O	465 ml
50x VB salts (Recipe 1)	10 ml
40% glucose	25 ml

Add agar in 465 ml of distilled water and autoclave for 20 min, at 121 °C. After cooling, add the salts and glucose gently
  10. Histidine/Biotin plates (Master plates for non R-factor strains)
 

*Used in tests for histidine requirement*

Ingredients	Per 500 ml
Agar	7.5 g
Distilled H <sub>2</sub> O	457 ml
50x VB salts	10 ml
40% glucose	25 ml
Sterile histidine (2 g per 400 ml H <sub>2</sub> O)	5 ml
Sterile 0.5 mM biotin	3 ml

Dissolve agar in the given concentration in distilled water. Autoclave each solution separately for 20 min. After cooling of solution, add each salt gently
  11. Ampicillin and tetracycline\* plates
 

*Master plates for the cultivation of strains containing the plasmids pKM101 and pAQ1\**

Ingredients	Per 500 ml
Agar	7.5 g
Distilled H <sub>2</sub> O	405 ml
50x VB salts	10 ml
40% glucose	25 ml
Sterile histidine (2 g per 400 ml H <sub>2</sub> O)	5 ml
Sterile 0.5 mM biotin	3 ml

Sterile ampicillin solution (4 mg/ml 0.02 N NaOH) 1.58 ml

\*Sterile tetracycline solution (4 mg/ml 0.02 N HCl) 0.125 ml

Dissolve agar in the given concentration in distilled water. Autoclave each solution separately for 20 min. After cooling of solution, add each salt gently

*\*Note: TA 102 is resistant to tetracycline. The shelf life of the plates is two weeks at 4 °C.*

## 12. Nutrient agar plates

*Used in tests for genotypes [Crystal violet sensitivity (*rfa*) and UV sensitivity (*AuvrB*)] and viability of bacteria*

Ingredients	Per 500 ml
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Nutrient agar	7.5 g
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Distilled H <sub>2</sub> O	500 ml
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Dissolve agar in the given concentration in distilled water. Autoclave separately for 20 min. Pour the cooled solution into the Petri plates

## References

1. Vijay, U., Gupta, S., Mathur, P., Suravajhala, P. and Bhatnagar, P. (2018). [Microbial Mutagenicity Assay: Ames Test.](#) *Bio-protocol* 8(6): e2763.