Microbial Mutagenicity Ames Assay

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Abstract

The Microbial mutagenicty Ames bioassay is a short term microbial assay performed in *vitro.* It consisting of several *Salmonella typhimurium/E.coli* different strains, which are used to evaluate the mutagenicity of different environmental carcinogens and mutagen. Ames assay is used to detect the revert mutations (revertants) which are present in these strains. While, these bacterial strains synthesize the essential amino acid by restoring their functional capacity, it is also used to detect the mutagenicity of different environmental samples such as drugs, dyes, reagent, cosmetics, waste water, pesticides and other substances which are easily solubilized in water and form micro-suspension with strain. This recipe is best applicable for researchers who wish to perform Ames assay in their research.

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Guidelines

Before starting our experiment:

1. Immediately after receiving the cultures, strain are genetically studied for their genotypes (R-factor, histidine necessity, *rfa* and *uvr* B mutation).
2. Before performing experiment, always a new set of frozen strains are to be prepared.
3. *Salmonella* is a pathogenic bacterium and so one needs to be careful while performing the experiments.

Protocol

Recipe for Ames Assay

Step 1.

1. **Stock solution and Media**

   1. Vogel-Bonner medium E (50X)

   Minimal agar

   Ingredients ***Per 500ml***

Warm distilled H_20 (45°C)	335 ml
Magnesium sulfate ($MgSO_4.7H_20$)	5 gm
Citric acid monohydrate	50 gm
Potassium phosphate, dibasic (anhydrous) (K_2HPO_4)	250 gm
Sodium ammonium phosphate ($NaHNH_4\ PO_4.4H_20$)	87.5 gm

Salts are added into the warm water in 2 liter flask. Place the flask on a hot plate. Each salt is allowed to mix entirely. Make the volume of the solution upto one liter. The solution is autoclaved for 20 min and the temperature is set at 121°C. The bottles are tightened after the solution gets cooled. Store in a glass bottle at 4°C.

2. ***0.5 mM histidine / biotin solution***

Mutagenic Bioassay

Ingredients	***Per 125 ml***
D-Biotin (F.W. 247.3)	15.45 mg
L-Histidine.HC1 (F.W. 191.7)	12.0 mg
Distilled H_20	125 ml

Dissolve the biotin in hot distilled water. The solution is autoclaved for 20 min and the temperature is set at 121°C. Store in a glass bottle at 4°C.

3. *Salt solution (1.65 M KCl + 0.4 M $MgCl_2$)*

S9 hepatic fraction

Ingredients	***Per 250 ml***
Potassium chloride (KCl)	30.75 gm
Magnesium chloride ($MgC1_2.\ 6H_20$)	20.35 gm
Distilled H_20 to final volume of 250 ml	

All the components are dissolved in water. The solution is autoclaved for 20 min and the temperature is set at 121°C. Store in a glass bottle at 4°C.

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

S9 hepatic fraction

Ingredients	***Per 250 ml***
0.2 M sodium dihydrogen phosphate ($NaH_2PO_4.H_20$)	30 ml (6.9 gm/250 ml)
0.2 M disodium hydrogen phosphate (Na_2HPO_4)	220 ml (7.1 gm/250 ml)

pH adjusted to 7.4. Sterilize the buffer by autoclaving for 20 min at 121ºC.

5. ***1M NADP solution (Nicotine Adenine Dinucleotide Phosphate)***

S9 hepatic fraction

Ingredients	***Per 2.5 ml***
NADP	191.5 mg
Sterile distilled H_2O	2.5 ml

NADP is dissolved in the distilled water and mixed by vortexing. Tubes are placed in an ice bath. This solution can be used until six months.

6. ***1M glucose-6-phosphate***

S9 hepatic fraction

Ingredients	***Per 5 ml***
Glucose-6-phosphate (G-6-P)	1.41 gm
Sterile distilled H_20	5 ml

Glucose-6-phosphate is dissolved in the 5 ml distilled water and mixed by vortexing. Tubes are placed in an ice bath. This solution can be used uptil six months.

7. ***Ampicillin solution (4mg/ml)***

Tests of ampicillin resistance

Master plates for R-factor strains

Ingredients	***Per 500ml***

Ampicillin trihydrate	0.4 gm
Sodium hydroxide (0.02 N)	50 ml

Ampicillin trihydrate is dissolved in the 50 ml of NaOH and mixed by vortexing. Tubes are placed in an ice bath.

8. ***Crystal violet solution (0.1%)***

Tests for crystal violet sensitivity (to confirm *rfa* mutation)

Ingredients	***Per 500 ml***
Crystal violet	0.05 gm
Distilled H_20	50 ml

9. ***Minimal glucose plates***

Mutagenic Bioassay

Ingredients	***Per 500 ml***
Agar	7.5g
Distilled H_20	465 ml
50X VB salts	10 ml
40% glucose	25 ml

Dissolve agar in distilled water. Autoclave both the solutions for 20 min. After cooling of solution, both the salts are added gently.

10. ***Histidine/Biotin plates***

Master plates for non R-factor strains

Tests for histidine requirement

Ingredients	***Per 500 ml***
Agar	7.5 gm
Distilled H_20	457 ml
50X VB salts	10 ml
40% glucose	25 ml
Sterile histidine (2 g per 400 ml H_20)	5 ml

Sterile 0.5 mM biotin	3 ml

Dissolve agar in the given volume of 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 strains containing the plasmids pKM101 and pAQ1*

Ingredients	***Per 500 ml***
Agar	7.5 gm
Distilled H_20	405ml
50X VB salts	10 ml
40% glucose	25 ml
Sterile histidine (2 g per 400 ml H_20)	5 ml
Sterile 0.5 mM biotin	3 ml
Sterile ampicillin solution(8 mg/ml 0.02 N NaOH)	1.58 ml
*Sterile tetracycline solution (8 mg/ml 0.02 N HC1)	0.125 ml

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

*TA 102 is resistant to tetracycline. The plates are discarded within two weeks of use.

12. ***Nutrient agar plates -*** Tests for genotypes

(a) Crystal violet sensitivity *(rfa)*

(b) UV sensitivity *(AuvrB)*

2. Tests for viability of bacteria

Ingredients	***Per 500 ml***
Nutrient Agar	7.5 gm
Distilled H_20	500 ml

Dissolve agar in the given volume of distilled water. Autoclave separately for 20 min. Pour the cooled solution into the petri plates.

13. S9 mix (Rat Liver Microsomal Enzymes + Cofactors)

Ingredients	***Standard S9 mix Per 25 ml***
Mice liver	1.0 ml (2%)
MgC12-KC1 salts	0.5 ml
1 M glucose-6-phosphate	0.125 ml
0.1 M NADP	1.0 ml
0.2 M phosphate buffer, pH 7.4	12.5ml
Sterile distilled H_20	9.86 ml

Each ingredient is added in reverse order in the solution. We should never allow refreezing the hepatic fraction.

Warnings

S.typhimurium is a pathogenic bacterium. While performing experiment, it is prudent to take necesary precaution every time and applying standard biosafety guidelines. All handling of chemicals should be performed in a chemical safety cabinet.

All contaminated material (e.g., test tubes, pipettes and pipette tips, gowns and gloves) should be properly autoclaved and disposed off properly.