APPENDIX

Reagents and Solutions used in Experimentation

1. Enzyme Assay:

Reagents:

(A) Diacetyl mono oxime: Add 2 g of the solid to about 60 ml of distilled water, add 2 ml glacial acetic acid, shake to dissolve with slight warming and make up to 100 ml with distilled water.

(B) Sulphuric acid - Phosphoric acid reagent: Add 150 ml of 85% phosphoric acid to 140 ml distilled water, mix well, add 30 ml of conc. sulphuric acid slowly with mixing.
2. Tissue Culture:

Solutions:

(A) Dulbecco's Phosphate Buffer Saline (PBS):

\[
\text{mg/l} \\
(i) \quad \text{NaCl} & 8000 \\
(ii) \quad \text{KCl} & 200 \\
(iii) \quad \text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O} & 1400 \\
(iv) \quad \text{KH}_2\text{PO}_4 & 200 \\
(v) \quad \text{Distilled water (make up to)} & 1000 \text{ ml}
\]

Sterilized by autoclaving at 15 lb for 15 min.

(B) Versen (EDTA): 2% solution

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<td>Ethylene-diamine-tetra acetic acid</td>
<td>200 mg</td>
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<td>Distilled water</td>
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Autoclaved at 15 lb for 15 min.

(C) Trypsine (1:250) 2.5% stock solution: 2.5 g Trypsin (1:250) was added to 100 ml of PBS and stirred on magnetic stirrer at 37°C for 1 to 2 hrs and centrifuged at 5000 rpm for 15 min to remove the unsoluble matter. It was sterilized by filtration through 0.22 μm membrane filter. Stored at -20°C.
(D) Working stock of TPVG:

(i) PBS 84 ml
(ii) 2.5% Trypsin 5 ml
(iii) 0.2% Versene 10 ml
(iv) 10% D-glucose 5 ml
(v) Penicillin 100 units/ml

Stored at -20°C.

(E) Trypan Blue (0.4%):

0.4 g of Trypan blue was dissolved in 100 ml PBS.

(F) Bouin's Fluid (Fixative):

(i) Formaline 250 ml
(ii) Saturated aq. solution of Picric acid 750 ml
(iii) Acetic acid glacial 5 ml

3. Reagents for SDS Polyacrylamide Gel Electrophoresis:

(A) Sample buffer:

(i) NaH$_2$PO$_4$ 0.34 g
(ii) Na$_2$HPO$_4$ 1.02 g
(iii) Sodium dodecyl sulphate 1.00 g

(iv) β-Mercaptoethanol 1.00 ml

(v) Bromophenol Blue 0.015 g

(vi) Urea 36.00 g

Dissolved and diluted to 100 ml with water.

Stored at 0-5°C.

(B) Gel buffer:

(i) NaH₂PO₄ 6.80 g

(ii) Na₂HPO₄ 20.45 g

(iii) Sodium dodecyl sulphate 2.00 g

Dissolved and diluted to 1 liter with distilled water.

Stored at 0-5°C.

(C) Acrylamide Gel (10% Gel):

(i) Acrylamide 22.2 g

(ii) N,N'-Methylenebisacrylamide 0.6 g

Stored at 0-5°C.

(D) N,N,N',N'-Tetramethylethylenediamine (THEDA):

Stored at room temperature.

(E) Ammonium persulfate solution:

Dissolved 100 mg ammonium persulfate in 15 ml distilled water.
(F) Fixative solution:

Prepared solution by combining:

- Methanol 400 ml
- Glacial acetic acid 70 ml
- Distilled water 530 ml

(G) Staining Reagent:

Prepared solution by dissolving:

- Brilliant Blue R 1.25 g
  (Coomassie Brilliant Blue R)
- Reagent F 500 ml
पं सुन्दरलाल शर्मा ग्रन्थागार

पं रविशंकर मुख्य विद्याध्याय, खरगुप्त (भ. यु.)

विलायक एक्स

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