APPENDIX
APPENDIX

A list consisting of the methods for the preparation of various reagents used is given below:

FIXATIVES

Zenker fluid

Stock solution was prepared by adding:
- Potassium dichromate 2.5 g
- Mercuric chloride 5.0 g
- Distilled water 100 ml

In 20 ml of this solution was added 1 ml of acetic acid just before use.

Bouins fluid

This was prepared by mixing the following:
- Saturated aqueous solution of picric acid 75 ml
- 40% formaldehyde 25 ml
- Glacial acetic acid 5 ml

Formaldehyde calcium

The fixatives was prepared by adding:
- Calcium chloride 2 g
- 10% formalin 10 ml
- H₂O 100 ml

The solution was made from formalin neutralized with marble chips.

3% Glutaraldehyde

To prepare 500 ml of 3% glutaraldehyde in 0.1 M of phosphate buffer, pH 7.4.

Took 60 ml of 25% solution of glutaraldehyde and made the vol to 250 ml with double distilled water. Add 250 ml of 0.2 M phosphate buffer pH 7.4 and store at 4°C.

Dichromate Calcium

Potassium dichromate 5 g
Calcium chloride - 1 g
Distilled water - 100 ml

1% Osmium tetroxide (1% OsO₄)

It is supplied as 1 g of the compound contained in ampules. A stock solution of 2% OsO₄ was prepared by dissolving 1 g in 50 ml of double distilled water. OsO₄ took some time (over night) to dissolve. Store at 4°C in tightly stoppered brown coloured bottle.

To a part of 2% OsO₄ solution added equal part of 0.2 M sodium cacodylate buffer to achieve 1% OsO₄ in 0.1M buffer solution. Prepare fresh before use.

Embedding media
1. Epon Resin 812 - 5 ml
   Araldite Resin 502 - 5 ml
   DDSA (Dodocenyl succinic anhydride) - 12 ml
   DMP (30 catalyst) - 0.66 ml
2. ERL 4206 - 10 g
   DER 736 - 6 g
   NSA(Nonenyl Succinic Anhydride)-26 g
   SI-DMAE (dimethylamino ethanol) - 0.4 g

STAINS

Iron haematoxylin

This stain was prepared by adding 250 mg of haematoxylin and 25 mg of sodium iodate to 47.5 ml of boiling distilled water. On cooling, 2.5 ml of absolute alcohol was added.

Eosine

One g of eosine in 100 ml of 90% alcohol.

Schiff's reagent

200 ml of distilled water was boiled and in it 1 g of basic fuchsin was dissolved. The solution was cooled to 50°C and was filtered. To the filtrate was added 20 ml of 1N HCl. After cooling to 25°C, 1 g of sodium metabisulphite was dissolved in it. The mixture was left in the dark for overnight. After 24 h the solution was decolorized with
activated charcoal and filtered. A clear solution was obtained which was stored in refrigerator in a dark coloured bottle.

**Carbol-Fuchsin**

Stock A = 3 g of basic fuchsin in 100 ml of 70% alcohol.

Stock B = (working solution)

10 ml of Stock A + 90 ml of 5% phenol + 12 ml formaldehyde + 12 ml acetic acid.

**Uranyl acetate**

Prepared a saturated solution by adding 450 mg of Uranyl acetate (Analar grade) to 10 ml of filtered 50% ethanol in a 15 ml centrifuge tube, shaken vigorously for 2 min and spun down to allow the excess of uranyl acetate to settle down. The solution is ready for use which may be stoppered and stored at 4°C.

**Lead citrate**

Added one half pellet of sodium hydroxide to 12 ml of double distilled water in a 15 ml centrifuge tube. Shaken well to dissolve. Added 50 mg of Lead citrate (Analar) and shaken well for 12 min and centrifuged, stoppered and stored at 4°C.

**Minimal Capacitation medium for sperm**

- NaCl (110 mM) - 642.95 mg/100 ml of distilled water
- NaHCO₃ (25 mM) - 210 mg/100 ml of distilled water
- CaCl₂ (1 mM) - 11.1 mg/100 ml of distilled water
- Na-Pyruvate (1 mM) - 11.1 mg/100 ml of distilled water

**BIOCHEMICAL REAGENTS**

**Acid ammonium molybdate reagent**

Ammonium molybdate = 25 g
10 N H₂SO₄ = 500 ml
(137.5 ml Conc H₂SO₄ in 362.5 ml distilled water)

These ingredients were mixed and 500 ml of distilled water was added to it.

Amino-naphthol sulphonic acid (ANSA)

15 g of sodium metabisulphite were dissolved in 200 ml of distilled water. To 195 ml of this solution, 0.5 g of 1-amino-2-naphthol-4-sulphonic acid and 5 ml of sodium sulphite, 20% solution was added. This solution was decolourized with 1 g of activated charcoal and was kept in dark for overnight. The colourless filtrate was stored in the refrigerator.

FeCl₃ colouring reagent for cholesterol

To 15 ml of conc H₂SO₄ was added 1 ml ferric chloride solution (10 mg FeCl₃ .6H₂O/l ml glacial acetic acid) agitating well. The vol was made 100 ml with Conc H₂SO₄. A clear pale yellow solution was obtained.

Cobalt nitrate - acetic acid reagent

Sol A : Prepared a saturated potassium sulphate in boiling water, filtered. Added 6.0 g of Cobalt nitrate and 0.8 ml of glacial acetic acid to the potassium sulphate solution and finally the vol was made 100 ml at 37°C.

Sol B : Saturated solution of sodium sulphate was prepared in the boiling water and kept at 37°C overnight.

Now 1.35 vol of tiethanolamine was made 10 vols with solution A and to this 7 vols of B was added. It should be prepared freshly before use.

α-Nitroso - β-Naphthol solution:

Stock solution 0.4% - 400 mg of α-Nitrosoβ- Naphthol was prepared in 100 ml absolute (96%) alcohol.

Stock solution was diluted with 96% ethanol by a factor of 12.5 before use.

Fatty acid standard - 0.028 g of palmtic acid in 50 ml of Dole's reagent.

Working standard: 1 ml of stock was diluted to 4 ml with Dole's reagent.
Cupric acetate pyridine reagent

5% aqueous cupric acetate sol was filtered and its pH was adjusted to 6.0 to 6.2 with the pyridine.

Dische diphenylamine reagent

1 g of diphenylamine was dissolved in 100 ml of glacial acetic acid. To this solution was added 2.75 ml of Conc sulphuric acid. This solution was made freshly each time.

Orcinol reagent

To 1 ml of 10% orcinol solution in water was added 40 ml of Conc HCl and 1 ml of 10% ferric chloride solution in water. This solution was freshly prepared each time.

Biuret reagent

CuSO₄ - 1.5 g
Na/K tartarate - 6 g
Distilled water - 500 ml
10% NaOH - 300 ml (30 g/300 ml)

The final vol of solution was made upto 1 litre.

Acid phosphatase substrate

424 mg of sodium diethylbarbiturate were dissolved in 20 ml of distilled water. To this solution was added 500 mg of sodium beta-glycerophosphate and 5 ml of 1N acetic acid. The total vol was made 100 ml with distilled water and pH was adjusted to 5.2 with 0.1N acetic acid or 0.1N NaOH and kept in the refrigerator.

Alkaline phosphatase substrate

Dissolved 2.5 g sodium beta-glycerophosphate and 2.12 g sodium diethylbarbiturate in 500 ml of distilled water. The pH of the solution was adjusted to 8.5 and stored in the refrigerator.

ATP-Mg mixture

88 mg of sodium salt of ATP (0.075M) and 40.5g of MgCl₂ (0.04 M) were dissolved in 5 ml distilled water.

Histidine, Tris-EDTA Buffer

In 5 ml of distilled water, 77.5 mg of histidine
(0.1M), 60.5 mg of Tris (0.1M), 18.6 mg of EDTA (0.01M) and 10.1 mg of MgCl₂ (0.01M) were dissolved.

Phosphate buffer (0.05M) (pH 7.0)

A) 0.2M NaH₂PO₄.2H₂O (31.21g in 1 litre)
B) 0.2M Na₂HPO₄.7H₂O (53.65g in 1 litre)

19.75 ml of (a) and 30.5 ml of (b) diluted to 200 ml for 0.05M AT pH 7.0.

DNSA

Dissolved 1 g of DNSA in 30 ml of distilled water. To this, 40 ml of 1 N NaOH and 30 g of Na/K tartarate was added and finally the vol was made to 100 ml.

Boric acid - NaOH buffer (0.05 M, pH 9.5)

1.545 g boric acid was dissolved in 500 ml distilled water and the pH was adjusted with NaOH (0.05M) (200 mg NaOH in 100 ml distilled water).

Citrate buffer (0.1 M, pH 6.2 and 0.067 M, pH 6.0)

A) 0.1 M solution of citric acid (2.101 g in 100 ml of distilled water)
B) 0.1 M solution of sodium citrate (2.941 g CH₂O Na .2H₂O in 100 ml distilled water)

For pH 6.2 : 7.2 ml of A and 42.8 ml of B were mixed and vol was made to 100 ml with distilled water.

For 0.067 M, pH 6.0 : 9.5 ml of A and 41.5 ml of B were mixed and vol was made to 100 ml with distilled water. 67 ml of this was diluted to 100 ml with distilled water.

Tris-HCl buffer (0.1 M, 0.2M, pH 7.6 and pH 9.0)

A) 0.2 M solution of tris (24.2g Tris in 1000 ml distilled water)
B) : 0.2 M HCl (7.294 ml HCl in 100 ml water).

For pH 7.4, 0.1 M : 50 ml of A and 41.4 ml of B were mixed and the vol was made to 200 ml with distilled water.

For pH 7.6, 0.2M : 50 ml of A and 38.4 ml of B were mixed and the vol was made to 100 ml with distilled water.
For pH 9.0, 0.1 M: 50 ml of A and 50 ml of B were mixed and vol was made to 200 ml with distilled water.

**Alcoholic resorcinol (0.1% solution)**

1 g thiourea and 250 mg of resorcinol was dissolved in 100 ml acetic acid.

**Glycine buffer (0.1 M)**

- Glycine: 7.505 g
- Sodium chloride: 5.85 g

The above ingredients were dissolved in 100 ml distilled water and the final vol was made to 1 litre with distilled water.

**Buffered substrate for LDH**

The substrate was prepared by mixing:

- Glycine buffer: 125 ml
- 0.1 N NaOH: 75 ml
- Sodium lactate solution (70%): 5 ml

**Dinitrophenyl hydrazine (DNPH) reagent**

200 mg DNPH was dissolved in hot HCl and the final volume was made to 1 litre with hot HCl.

**0.4N Sodium hydroxide**

1.6 g sodium hydroxide was dissolved in water and the final vol was made 100 ml.

**0.1 M phosphate buffer (0.1M, pH 7.4)**

**Stock solution**

- **A)** 0.2 M monobasic sodium phosphate (Na H₂PO₄, H₂O)
  - 2.76 g in 100 ml of distilled water.

- **B)** 0.2M dibasic sodium phosphate
  - 5.36 g of Na₂HPO₄. 7H O or
  - 7.6g of Na₂HPO₄.12 H₂O
  in 100ml of distilled water.

For pH 7.4: 19 ml of A and 81 ml of B were mixed and vol was made to 200 ml with distilled water.

**Potassium bromide-bromine reagent**

100ml water was saturated with bromine and 12g of potassium bromide was dissolved in it.
Dinitrophenylhydrazine reagent for ascorbic acid

The reagent was prepared adding:
- 2,4-dinitrophenylhydrazine = 2 g
- Thiourea = 250 mg
- Copper sulphate = 30 mg

These ingredients were dissolved in 100 ml of 9N H$_2$SO$_4$.

Sodium periodate solution (0.2M)

4.28 g of sodium periodate (meta) was dissolved in 9M orthophosphoric acid and final vol was made 100 ml.

9M Phosphoric acid

88.2 ml of orthophosphoric acid was taken and vol was made 100 ml with distilled water.

Sodium arsenite solution

10 g of sodium arsenite was dissolved in 100 ml of 0.5 M sodium sulphate solution prepared in 0.1 N H$_2$SO$_4$. (0.28 ml Conc H$_2$SO$_4$ was taken and vol was made 100 ml with distilled water).

0.5 M Sodium sulphate solution

7.1 g of sodium sulphate was dissolved in 0.1N H$_2$SO$_4$ and final vol was made 100 ml.

Triethanolamine buffer (0.1M)

1.86 g triethanolamine hydrochloride/100 ml of water. pH 7.6 was adjusted with 1N NaOH.

Phosphate buffer (0.1M)

A : 1.361 g of KH$_2$PO$_4$/100 ml of water
B : 2.282 g K$_2$HPO$_4$.3H O/100 ml of water mix A and B in the ratio of 12:88

Hydroxylamine hydrochloride (HAHCL) reagent -2M/litre. mix equal vols of HAHCL reagent and water freshly before use.

Hydroxylamine reagent for HMG-CoA -2M/litre. mix equal vol. of HA HCl reagent and NaOH (4.5 M) freshly before use.

Ferric chloride reagent for HMG-Co-A.

Dissolve 5.2 g of TCA and 10 g FeCl$_3$ in 50 ml of 0.65 M HCl and dilute to 100 ml with the later.
Substrate (Ediol) for Tq.lipase - 1 ml of 50% coconut oil was dissolved in 9 ml of 50% gum arabic solution.

Kreb's Ringer buffer (pH 7.4)
1) 0.9% NaCl (0.154 M)
2) 1.15% KCl (0.154 M)
3) 1.22% CaCl₂ (0.11 M)
4) 2.11% KH₂PO₄ (0.154 M)
5) 3.82% MgSO₄·7H₂O (0.154 M)
6) 1.3% NaHCO₃ (0.154 M)

To 100 parts of (1) 4 parts of (2), 3 parts of (3), 1 part of (4), 1 part of (5) and 21 parts of (6).
Buffer prepared was gassed for 10 min. with Carbogen (95% O₂ and 5% O₂), pH adjusted to 7.4, glucose 5% sol. added in the preparation of 0.4 ml to 19.6 ml of buffer.

Tris-HCl (1.35M) - 16.348 g of Tris was dissolved in 100 ml of 0.145 M NaCl, and the pH was adjusted to 8.5.

GOT buffer
In a 100 ml volumetric flask, was added 29.2 mg of alpha ketoglutaric acid and 2.66 g of dl aspartic acid. To these was added a small quantity of 1 N sodium hydroxide solution to dissolve them and the pH was adjusted to 7.4. The vol was made up to 100 ml by adding phosphate buffer, pH 7.4. One drop of chloroform was added as a preservative. The buffer was refrigerated until used.

GPT Buffer
In a 100 ml volumetric flask was added 29.2 mg of alpha ketoglutaric acid and 1.78 g of alanine. The ingredients were dissolved in a small amount of 1 N sodium hydroxide solution and the pH adjusted to 7.4. The vol was made up to 100 ml with phosphate buffer, pH 7.4 and one drop of chloroform was added as a preservative. The buffer was stored in the refrigerator until used.

2,4 Dinitrophenyl hydrazine reagent for transaminases
198 mg of dinitrophenyl hydrazine was dissolved and the vol made up to one litre with 1N HCl.
1N HCl – 86.2 ml Conc HCl diluted to 1 litre with distilled water.

2/3 N sulphuric acid

18.5 ml of Conc. sulphuric acid was added to water and the vol made upto one litre.

Glucose solution (0.01 M)

17.1 mg of glucose was dissolved in 5 ml of distilled water.

Alkaline-copper reagent

Sol A 5% copper sulphate
Sol B 70 g of sodium carbonate, 22 g of sodium bicarbonate and 26 g of sodium tartarate were dissolved in water and the vol was made upto one litre with an excess of water. One part of A and nine parts of B were mixed before use.

Tungstic acid:

A) Sulphuric acid (0.15N) : 4.17 ml of Conc sulphuric acid was diluted to one litre with water.

B) Sodium tungstate (2.2%) : 22 g of sodium tungstate was dissolved in water and made upto one litre. Equal parts of (A) and (B) were mixed immediately before use.

Phosphomolybdic acid

A) Brominated sodium molybdate: 300 g of sodium molybdate were mixed in 500 ml of water. To this solution were added two drops of bromine and the final vol made upto one litre with water.

B) Sulphuric acid (25%): 135 ml of Conc sulphuric acid was added to 500 ml of water and the vol made upto one litre with more water to get 25% sulphuric acid (by weight) solution.

In a one litre volumetric flask, 500 ml of clear supernatant of (A) and 225 ml of 85% phosphoric acid and 150
ml of (B) were added. The bromine was removed from the solution by blowing air into it and 75 ml of glacial acetic acid was added. The vol was made up to one litre with water.

*Sulphanilic acid solution (0.1%)*

One g of sulphanilic acid was diluted to one litre in 0.18 N hydrochloric acid.

**0.18 N HCl :** 15.5 ml Conc. HCl diluted to litre with water.

*Diazotizing reagent*

0.3 ml of the 0.5% sodium nitrite solution was added to 10 ml of the 0.1% sulphanilic acid solution. The solution was made 30 min before use.

**Bloor's reagent**

95% ethyl alcohol and diethyl ether were mixed in a ratio of 3:1.

**Biuret reagent for serum proteins (Full strength and half strength):**

A fresh solution of Biuret reagent was prepared every time by adding 5 parts of 25% sodium hydroxide to one part of 2% copper sulphate. This gave full strength Biuret. For half strength, 25 ml of this was made up to 50 ml in a volumetric flask with water.

**Tris-HCl (1.35 M)** - 16.348 g of Tris was dissolved in 100 ml of 0.145 M NaCl. and pH was adjusted to 8.5.

**Sodium phosphotungstate**

11.25 g/100 ml of water was mixed 40 ml of 1 M and the vol was made to 250 ml with water.

**Sodium dodecyl sulphate (10%)**

10 g of sodium dodecyl sulphate (SDS) was dissolved in 100 ml of 0.15 molar sodium chloride (NaCl) and then the pH was adjusted to 9.00.

**0.15M NaCl - .8775 g/100 ml**

**1N CH₃COOH**

To 57.7 ml glacial acetic acid was added 942.3 ml distilled water.
6.25 M KOH
Dissolved 35 g KOH in 100 ml distilled water.
MgCl$_2$ - 40.68 g/100 ml
H$_2$SO$_4$ - 0.1 ml of Conc. H$_2$SO$_4$ in 3.5 ml of distilled water.
Tris - HCl buffer (pH 6.9) for measurement of intestinal uptake

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>140 mM - 818 mg/100 ml</td>
</tr>
<tr>
<td>KCl</td>
<td>6 mM - 447 mg/100 ml</td>
</tr>
<tr>
<td>Tris buffer</td>
<td>4 mM - 48.45 mg/100 ml</td>
</tr>
<tr>
<td>Calcium</td>
<td>1 mM - 11 mg/100 ml</td>
</tr>
</tbody>
</table>

Scintillation fluid (Bray's fluid)

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>60 g</td>
</tr>
<tr>
<td>PPO</td>
<td>4 g</td>
</tr>
<tr>
<td>POPOP</td>
<td>0.2 g</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>20 ml</td>
</tr>
<tr>
<td>Glacial acetic acid</td>
<td>20 ml</td>
</tr>
<tr>
<td>Absolute methanol</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

and final vol was raised to one litre with 1,4 dioxan (860) ml.

10 mM Sodium maleate buffer, (pH 6.8)

Stock Sols

A) 0.2 M sol. of acid sodium maleate
(8 g of NaOH + 23.2 g maleic acid or
19.6 g maleic anhydride in 1000 ml)

B) 0.2 M NaOH (8g in 1000 ml
50 ml of A + x ml of B diluted to a total of 200 ml
x = 44.4 ml

1 mM Tris - 50 mM mannitol buffer (pH 7.4)
12.114 mg Tris + 910.85 mg mannitol in 100 ml

50 mM Sodium maleate buffer pH 6.5 (containing 0.02% sodium azide)

A) 0.05 M sol of acid sodium maleate
(200 mg NaOH + 830 mg maleic acid in 100 ml)
B) 0.05 M NaOH (200 mg NaOH in 100 ml)
50 ml of A + x ml of B diluted to a total of 200 ml
x = 40 ml pH = 6.5
Add 40 mg (0.02%) sodium azide to final vol.

Buffered substrate for alkaline phosphatase in BBM

- Containing $5.5 \times 10^{-3}$ M p-nitrophenyl 1 phosphate (0.5 M, with pH 10.5).
- 375 mg of glycine
- 10 mg of MgCl$_2$
- 165 mg of p-nitrophenyl phosphate (sodium salt)

were dissolved in 42 ml of 0.1 N NaOH and diluted to 100 ml with distilled water. pH was set to 10.5.

p-Nitrophenol standard

- 13.9 mg p-nitrophenol was dissolved in 100 ml of distilled water (1 ml = 1 μmole p-nitrophenol).

Buffered substrate for leucine aminopeptidase

- Dissolved 20 mg of L-leucyl-β-naphthylamide in 100 ml of 0.2 M phosphate buffer, pH 7.0
- Sodium nitrite (0.1%)

- Dissolved 100 mg of NaNO in 100 ml of distilled water.

Ammonium sulfamate (0.5%)

- Dissolved 500 mg of ammonium sulfamate in 100 ml of distilled water.

Colouring reagent for leucine-aminopeptidase

- 50 mg of N-1-naphthylethylene diamine dihydrochloride was dissolved in 100 ml of alcohol.

β-naphthylamine standard

- Stock: 10 mg/100 ml in 2N HCl.
- Working standard (100 μg/ml): 1 ml of stock standard was diluted to 10 ml with 2N HCl.

Buffered substrate for glucose-6-phosphatase in BBM

- Dissolved 40 mg potassium salt of Glucose-6-phosphate in 2 ml of 0.1 M citrate buffer, pH 6.5.
10% TCA
Dissolved 10g trichloroacetic acid in 100 ml distilled water.

Sorenson glycine buffer for LDH in BBM (0.1 M):
7.505 g glycine and 5.85 g NaCl were dissolved in one litre of distilled water.

NAD - Dissolved 5 mg/ml of water.

Buffered substrate
Sodium lactate (pH 10.0): 5.8 ml of 60% sodium lactate was mixed in 125 ml of 0.1 M glycine buffer and 75 ml of 0.1N NaOH.
0.4 N NaOH: 16 g NaOH/litre

Colouring reagent: 200 mg DNPH is dissolved in 1N HCL. Then it was cooled and vol was made to one litre with 1N HCL.

Phosphate buffer (0.05 M) (pH 6.0)
A) 0.2 M NaH$_2$PO$_4$·2H$_2$O (31.21 g in 1 litre)
B) 0.2 M Na$_2$HPO$_4$·7H$_2$O (53.65 g) in 1 litre
21.92 ml of (A) and 3.07 ml of (B) diluted to 200 ml for 0.05 M at pH 6.0

Maltose solution (0.2 M)
Dissolved 684 mg of maltose in 10 ml of 50 mM phosphate buffer.

Lactose solution (0.2 M)
Dissolved 684 mg of lactose in 10 ml of same buffer.

Sucrose solution (0.2 M)
684 mg sucrose were dissolved in 10 ml of same buffer.

Tris buffer (0.5 M) (pH 7.0)
Dissolved 30 g Tris in about 200 ml of water and pH was adjusted to 7.0 by adding 1N HCl, vol made to 1 litre with distilled water.

Glucose oxidase (GOD reagent)
A) Tris (1M) 12.1 g/100 ml Adjust pH 7.2 with 1N HCl or Conc HCl.
B) p-OH benzoic acid (para-hydroxy benzoic acid): 0.276 g/20 ml. Adjust pH 7.0 with 2N NaOH.

C) 4-amino antipyrene - 16.4 mg

D) Peroxidase - 1 mg

E) glucose oxidase - 0.2 ml (2 mg)

First adjust the pH of sol. A and B separately and then add c, d, e and make the total vol 200 ml with D.W. Mix all the solutions freshly just before use.

Standard glucose
Dissolved 180 mg of analytical grade glucose in 10 ml distilled water.
0.1 ml of stock solution was diluted with 10 ml water to get the working standard solution.

Modified Lowry method for myelin protein

Reagents:
A) 5% SDS (Sodium dodecyl sulphate) in 0.5 N NaOH
   Make fresh by mixing equal quantities of 10% SDS and 1 N NaOH (some brands of SDS will precipitate in 0.5 N NaOH). Store in plastic but not more than 1 week.

B) Copper tartarate solution
   50 ml each of 2% Na tartarate
   1% CuSO₄·5H₂O
   2.5 ml of 1 N NaOH.
   (Shelf life one month).

C) Folin phenol Reagent : 1 N
   Dilute 2 N,1 : 1 with Distilled water

D) 2% Na₂CO₃

E) Standard BSA - 2mg/ml of H₂O.
   Pyruvate Standard - 11 mg sodium pyruvate/100 ml of buffered substrate.

Warren's formula for calculations of ganglioside sialic acid

\[
0.07 \times OD \times 50 = \mu\text{mole/g}
\]


