جامعة الملك سعود
King Saud University
كلية العلوم الطبية التطبيقية
قسم علوم المختبرات الاكلينيكية

Biochemistry 2 Booklet

CLS 331

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Chemicel list biochemistry2

CLS 331

Name	Unt	EXPIRATION DATE
Phosphate buffer (ph:6.7)	gm	
Starch	gm	
Iodine	Gm	
Sodium chloride	gm	
Phosphate buffer(ph:6.7)	Gm	
Starch	Gm	
Sodium chloride	Gm	
Sodium hydroxid	Gm	
maltose	Gm	
3,5Dinitrosalicylic acid	gm	
Sodium potassium tartarate	Gm	
Disodium hydrogen phosphate	Gm	
Potassium dihdrogen phosphate	Gm	
Yeast suspension	Gm	
Glucose	Gm	
Sodium nitroprussside	Gm	
Ammonium hydroxide	Gm	
Ammonium sulphate	Gm	
Sodium sulphite	Gm	
Sodium hydroxide	gm	
2,4dinitrophenylhydrazine hydrochloride	Gm	
Hydrochloric acid	Gm	
piperidine	Ml	
Trichloracetic acid	ml	
Silica gel (20×20cm)	Box	
Butanol	Ml	
Ethanol	Ml	
Alanine	Gm	
argnine	Gm	
proline	Gm	
Tyrosine	Gm	

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Ninhydrin	gm	
Acetic acid	gm	
Hydrochloric acid	ml	

Name	**Unt**	**EXPIRATION DATE**
Egg albumin	gm	
Copper sulphate	Gm	
Sodium potassium tartarate	Gm	
Sodium hydroxide	Gm	
Potassium iodide	Gm	
Sodium carbonate	Gm	
Folin-ciocalteau reagent	ml	
Frish milk	Ml	
Saturated litmus solution	ml	
Pancreatin	Gm	
Calcium hydroxide	Gm	
Olive oil	Ml	
Sodium cholate	Gm	
Pancreatin	Gm	
Phenolphthalein	Gm	
Sodium hydroxide	Gm	
Urease(type 111 powder,sigma)	Gm	
Sodium edetate	Gm	
Phenol	Gm	
Sodium nitroprusside	Gm	
Sodium hydroxide	Gm	
Sodium hypochlorite	gm	
Ammonium molybdate	Gm	
Copper sulphate	Gm	
Sodium acetate	Gm	
Acetic acid	ml	
Metol	Gm	
Sodium sulphite	gm	
Trichloracetic acid	Gm	
Ppotassium dihydrogen phosphate	Gm	
Phosphorus acid	gm	

Expermint:1

The hydrolytic activity of salivary amylase on starch

Reagent :

1) phosphate Buffer(0.1M , ph (6)

Ready solution Or a powder (Dissolve in large Erlenmeyer flask(1L)
Dilute to mark by D.W .

2) Buffered starch substrate (0.5% in phosphate buffer)
a)5ml phosphate buffer (ph 6)+0.5 gm starch **(Max)**
b)100ml boiling phosphate buffer **(ph 6)+ (a) (Very max +boiling)**
c)27.5 ml (b) 50ml phosphate buffer (ph 6) **(Very max)**

3) Iodine solution (0.1N in 3% potassium iodide (KI)
a) 30gm potassium iodide + 1L D.W
b) (0.1N)Iodine : N= V \ m.wt *1L

$$0.1 = V \backslash 127 * 1L$$

$$V = 0.1 * 127 = 12.7 \text{ gm}$$

(12.7gm Iodine + 1L potassium iodide)

4) Iodine solution (0.005N in 3% KI) :

(1ml(0.1N)Iodine + 20ml (3%) KI)

5)Nacl (1%):

(1gm Nacl +100ml D.W)

Glass Ware :

2) pipette :

plastic (2)

1ml : (2)

2ml : (2)

5ml : (1)

3) Tubes (4).

4) Wooden sticlts .

5) Spottory plates.

6) Mechnecl Pipette .

Machine:

1) Water bath : a) 37c b) 95c

Expermint:2

Quantitative determination of amylase activity

Reagent:

1) Alkaline reagent :

1gm (3,5 dinitrosalicylic acid)+ 1gm sodium potassium tartarate + 30gm sodium hydroxide + 1L D .W

2) 1% Maltose :

(1gm Maltose + 100ml D.W)

4) Phosphate buffer (0.1M) ph : 7 .

(See Expermint:1)

5) Buffered starch substrate (0.5% in phosphate buffer)

(See Expermint:1)

6) Nacl (1%):

(1gm Nacl +100ml D.W)

7) (2N) NaOH :

N= V \ m.wt ×1L

2=(V \ 23+16+1)× 1L

2=(V \ 40) × 1L

V = 2×40 = 80 gm

(80 gm NaOH + 1L D.W)

Glass Ware :

1) pipette : 1ml (3)\ 10ml(2) \5ml (2)

2)Tubes (8).

3) Mechnecl Pipette .

4)Rax.

Machine:

1)Water bath :a) 95c

2) Vortex .

3) Spectrophotometers.

Expermint:3

The production of pyruvate and acetaldehyde during the fermentation of glucose by yeast.

Reagent:

1) (0.5 M) Disodium hydrogen phosphate (Na_2HPO_4).

(14.19gmNa_2HPO_4 + 100ml D.W)

2)(0.5M) potassium dihydrogen phosphate (KH_2PO_4).

(13.19 gm (KH_2PO_4)+100ml D.W)

3) Yeast suspension (10% in Na_2HPO_4) . **(Fresh)**

(10 gm yeast suspension +100ml (0.5M) Na_2HPO_4)

4) Yeast suspension (10% in KH_2PO_4) . **(Fresh)**

(10 gm yeast suspension +100ml (0.5M) KH_2PO_4)

5) Yeast suspension (10% in Water) **.(Fresh)**

(10gm Yeast suspension + 100m mlD.W)

6) Glucose (10%) .

(10gm Glucose + 100 ml D.W)

7)Sodium nitroprusside (5%) **.(Fresh)**

(5 gm Sodium nitroprusside + 100ml D.W)

(In Dark bottal)

8)Sodium hydroxide (10%) .

(10gm NaOH + 100ml D.W)

9)Piperidine (3%) **.(Fresh)**

(3gm Piperidine +100mlD.W)

10) Trichloracetic acid (10%).

(10 gm TCA + 100 ml D.W)

11) Ammonium sulphate (1\2 inch).

(1\2 inch = 0.5 gm Ammonium Sulphate)

In 2 tubes (15ml size).

12) Ammonium hudroxide .

(Ready in bottal)

13)2,4-Dinitrophenylhydrazine hydrochloride solution in 2N hydrochloric acid .

a) (2N) HcL : ***(wright on bottal(Hcl)M=63.01 \1L= 1.19 Kg)***

Wt = Vol × Sp.qvarity × 36.46

= 1000 × 1.19 ×0.364

= 433.16

M =Wt\ Mwt = 433.16 \ 36.46 = 11.88

M × V = M '× V'

11.88 × V = 2 × 1000

V = 2 × 1000 \11.88

V = 168.3

(168.3 ml + 1L D.W)

b) (4Gm)2,4-Dinitrophenylhydrazine **+** (2N) Hc

14) Sodium Sulphite : take abawder .

Glass Ware :

1) pipette : plastic (1)

2)Tubes 15ml (2) \with centrifuge (2).

3) Mechnecl Pipette .

4) Rax.(2).

Machine:

1)Water bath :a) 37c

3) centrifuge.

2) Spectrophotometers.

4) Vortex .

Expermint:4

Identification of amino acids by TLC using silica gel plates

Reagent:

1)Solvent :

(7ml Butanol – 2ml acetic acid – 2ml Water)

2) Silica gel .***(Fresh)***
(Placed in oven When (105C) For 45 **Minutes*).***

3) Amino acid in (0.1M) Hcl . ***(wright on bottal(Hcl M=63.01 \ 1L= 1.19 Kg)***

Wt = Vol × Sp.qvarity × 36.46

= 1000 × 1.19 ×0.364

= 433.16

M =Wt\ Mwt = 433.16 \ 36.46 = 11.88

M × V = M '× V'

11.88 × V =0.1 × 1000

V= 0.1 × 1000 \11.88

V = 8.41

(8.42 ml HcL + 1L D.W)

A) Alanine (5M)in (0.1M)HcL

M=(V \ m.wt) ×1L

5 = (V\89.09)×1L

V= 89.09 ×5 =445.45gm

(445.45gmAlanine + 1L (0.1M)HcL)

B)Argnine (5M)in (0.1M)HcL

M=(V \ m.wt) ×1L

5 =(V\174.2)×1L

V= 5 × 174.2 =871 gm

(871 gm Argnine + 1L (0.1M)HcL)

C)Proline(5M)in (0.1M)HcL

M=(V \ m.wt) ×1L

5 = (V\ 115.3) ×1L

V = 5 × 115.3 = 576.5gm

(576.5 gm Proline +1L (0.1M)HcL)

D) Tyrosine (5M) in (0.1M)HcL.

M=(V \ m.wt) ×1L

5= (V \181.19) × 1L

V= 5× 181.19 = 905.95 gm

(905.95 gm Tyrosine +1L (0.1M)HcL)

4) Unknown: **(1)**

(1ml alanine + 1ml arginine +1ml tyrosine).

Unknown: **(2)**

(1ml proline + 1ml argnine)

Unknown: **(3)**

(1ml proline + 1ml alanine + 1ml tyrosine)

Unknown: **(4)**

(1ml alanine + 1ml tyrosine)

5) Ninhydrine (o.3%)(N_4) .

(0.3 gm (N_4) + 100ml Butanol + 3ml acetic acid)

(In Location spray)

Glass Ware :

1) Micro pipette (1-5).
2) Tips .

Machine:

1) Hair dresser.

Expermint:5

Quantitative Determination of protein by the biuret & the lowry Reactions .

Reagent:

1) Protine Standard (5mg \ml) .

(5 gm albumin + 100ml D.W)

2) Biuret reagent .

a)(3gm) Copper sulphate + (9gm)Sodium potassium tartarate.

b) Sodium hydroxide (0.2N)

M=(V \ m.wt) ×1L

0.2 = (V\23+16+1) ×1L

0.2 (V\ 40)×1L

V= 0.2 × 40 = 8 gm

(8 gm NaOH + 1L D.W)

c) Potassium Iodide (5 gm)

d) 250 ml (0.2N)NaOH + (a)+ mix well in Machine + (c) + mix well in Machine + 250 ml NaOH+ placed in Flask standard 1L + Dilute to mark by D.W .

3) Unknown: **(1)**

In Class ***:(9.6 ml Protine standard +30.4ml D.W)***

In *finel exam* :(**160ml protine standard+ 40ml D.W)**

4) Alkaline reagent **(1)** : (2% Na_2CO_3 in 0.1N NaOH)

a) NaOH (0.1N) N=(V \ m.wt) ×1L

0.1= (V\ 1+16+23) ×1L

V = 0.1 ×40 = 4 gm ***(4 gm + 1L D.W)***

b) 2 gm (Na_2CO_3) + 100 ml (0.1M) NaOH

5) Alkaline reagent **(2)**(0.5% $CuSO_4$ in 1% Na_1K tartrate) **.(Fresh)**

a) Copper sulphate 0.5% (0.5 gm $CuSO_4$ + 100ml D.W)

b) Sodium potassium tartrate 1% (1 gm Na_1K tartrate + 100ml D.W)

c) 50 ml (a) + 50 ml (b) **(Fresh)**

6) Alkaline reagent **(3) (Fresh)**

50 ml Alkaline reagent **(1)** + 1ml AlKaline reagent **(2- c)**

7) Folin Ciocalteau reagent .

(100ml Folin Ciocalteau reagent+ 100ml D.W)

8) Protine standard .

(0.2 gm Albumin + 100ml D.W)

9) Unknown: **(2)**

(50 ml Protine standard + 50 ml D.W)

Glass Ware :

1) pipette : 5ml (3) \2ml (4) \1ml (4)

2)Tubes 15ml (8) \smal(8).

3) Mechnecl Pipette .

4) Rax .

Machine:

1)Water bath :a) 37c

2) Spectrophotometers.

3) Vortex .

Expermint:6

Enzymatic Digestion of fat by pancreatic lipase .

Reagent:

1)Homogenised whole milk preferably**(*fresh.)***

2) saturated Litmus solution :

(0.5gm Litmus + 100ml D.W) Or (ready in bottal)

3)Pancreation solution :

a) unboiled : 50ml Ethanol apselaet +50ml ml water +0.8 gm pancreatin (pH = 8)

b) boiled : 50ml (a) in water baath about 10 minutes.

4) Calcium hydroxide (1%) .

1gm Calcium hydroxide + 100ml D.W .

Glass Ware :

1) Pipeate : 1) blastic pip[eate (2) .2ml (2) . 1m (1) .

2) Test tubes (2) .

3) Mechnecl Pipette .

4) Rax .

Machine:

1)Water bath.45c

Expermint:7

Effect of Bile salts on pancreatic Lipase activity .

Reagent:

1) Vegetable oil (olive): or any oil
2) Sodium cholate solution .
   10 gm sodium cholate + 100ml D.W
3) D.W
4) Pancreatin solution (Lipase) .
   See Expermint (6) .(unboiled)
5) Phenolphtalein indicator (1%) .
   2gm phph + 200ml ethanol + 120ml D.W
6) Sodium hydroxide (0.05N) .
   M=(V \ m.wt) ×1L
   0.05 = (V\23+16+1) ×1L
   0.05 =(v\ 40)×1L
   V = 0.05 × 40 = 2gm
   (2gm NaOH + 1L D.W)

Glass Ware :

1) Pipeate : 1) blastic pip[eate (1) .2ml (1) . 1m (3) .

2) Test tubes (2) .

3) Mechnecl Pipette .

4) Flask (2) .

5) burette .

6) Funl .

Machine:

1) Water bath 45 c.

Expermint:8

Dethermination of serum urea

Reagent:

1) Buffered urease solution :

a) In large Erlenmeyer flask 100ml 1gm sodium edetate+ 80 ml D.W(pH: 6.5) .

b) (a) + 0.1 gm urease . Diulte to mark by D.W.

2) Phenol-sodium nitroprusside solution.

In large Erlenmeyer flask 1L 50 gm phenol + 0.25 gm sodium nitroprusside + Diulte to mark by D.W .

3) Sodium hydroxide – sodium hypochlorite (NaOcl)

25gmNaOH + 2.1 gm sodium hypochlorite + 1L D.W

4) Urea solution .

0.12 gm urea + 100ml ……

Glass Ware :

1)Pipeate : 1) blastic pip[eate (1) .2ml (1) . 1m (3) .

2) Test tubes (2) .

3) Mechnecl Pipette

Machine:

1)Water bath 37 c.

2) Spectrophotometers.

Expermint:9

The colorimetric estimation of inorganic phosphate .

Reagent:

1)Ammonium molybdate (5%) .

5gm Ammonium molybdate + 100gm D.W .

2) Copper acetate buffer (PH: 4).

a) (2N) acetic acid : ***(wright on bottal($HC_2H_3O_2$)M=60.05 \1L= 1.05 Kg).***

Wt = Vol × Sp.qvarity × 60.05

= 1000 × 1.05 × 60.05 \ 100

= 630

M =Wt\ Mwt

M = 630 \ 60 .05 = 10.49

M × V = M '× V'

10.49 × V = 2 × 1000

V = 2× 1000\ 10.49 = 190.6

(190.6 ml $HC_2H_3O_2$ + 1L D.W)

b)2.5gm copper sulphate + 46 gm sodium acetate .

c) (a) + (b) **3)**Metol – sodium sulphate solution **.(Fresh)**

a) sodium sulphite (10%) .

10gm sodium sulphite + 100ml D.W .

b)2gm Metol + **(a)** . **(in dark bottle)**

4)TCA (5%) :

(5 gm +100ml D.w) .

5) stock phosphate solution .

a) 0.438 gm potassium dihydrogen phosphate (pdp) + 100ml D.W

b) 0.1 gm phosphorus acid + **(a)** .

6) Working phosphate .

1ml stock phosphate solution + 100ml 5% TCA .

7) Unknown :

In class :**(50 ml working phosphate + 50 ml 5% TCA)**

In Finel exam : **(80 ml working phosphate+20ml 5%TCA)**

Glass Ware :

1) Pipette : 1ml (6) 2ml (1)

2) Test tubes (7) .

Machine:

1) Spectrophotometers