Storage Effects on galactosidase

7/14/49.

32 hour cells from 42 Lac 9:35 to 2:30

Assay is azide.

A. 1 ml cells 1 ml buffer B. water. Incubate

C " " D. " Refrigerate

	Initial Di	Initial Df.	Final Di	Final Df. 10 m.	Δ.
A	059	472	059	730	
B	061	242	061	109	
C	056	930	056	590	
D	060	241	060	160	

a). Note irregular excess of buffer treated cells over water treated. Buffer was M/10 Na. pH 7.5

Is activation related to Na^+?, λ? Assay in K buffer.

7/14/49.

PM. Harvest 10 hr. cells from Y2 Lac.

Dilute equal volume ē a) water b) NaP M/5 pH 7.5 c) KP do.
d) NaCl M/5 e) Sucrose M/5

.1 ml samples assayed.
β^{30} -10^{25}

Di H_2O 10 min.	084.	274	
a	075	158.	
b	042	>750 [5 mins].	✓
c	040	>750 [5 mins].	✓
d	066	410	
e	071	375	

[phosphate buffers, which also permit lysis, are most effective in augmenting activity.
pH effect? concentration? Measure pH's.]

verify lysis by uv absorption of supernatant.

Suspensions A and B contain ca. 1.5 and 2.2 mg/ml respectively. [For ≐ .1 mg, use $\frac{1}{15}$ ml for A and $\frac{1}{22}$ ml for B.]

Assay .05 ml each.

	D_i	D_f	Δ_{cor}
A	018	18.4	155
B	030	430 (5 mins).	390 × 4
Blank	001	014.	

−013 for substrate + 10% for dilution.

B) .11 mg had activity of $\frac{20}{5} \times 4 = 16u$. ≐ 150u/mg = full activity of the cells dried.

A) .075 mg had 1.5 u. ≐ 20u/mg. ≐ full activity, not augmented.

Differences between treated and untreated cells persist on drying.

?? Can inactive, cell-free or dried preparations be activated?

Sediment A and B. Resuspend sediment in 5 ml H_2O (= 1) and keep supernatant (= 2). B2 is much more opalescent than A1.

Save samples; also mix A1, A2 etc. 1:1 = NaP M/5.

Incubate $3^{30} \rightarrow 5^{30}$

Test .1 ml samples A1, A2 and A1P, A2P.

	Pi	
A1	040	155 10
A2		N 3 20
B1	068	530 5
B2		470 6
A1P	016	140 10
A2P		099 20
B1P	030	300 5
B2P		260 6

Y10 and Y70 grown on lactose. Incubate 1:1 with water, buffer M/10.

a) 4:30 — 7:00 Assay.

b) K-12 grown on lactose. Incubate 1:1 with water, buffer, etc.

K-12 / glucose [KG]. water, M/10 buffer.

1:1:1 lactose, 2%, water, M/100 buffer, M/10 buf.

	Di.		
KG O	062		
KG P	041		
KG-L-O	032	neg.	10m.
L-P	030		
L-P	027		
KL-O	139	371	6M
KL-P	111	520	6M
KL-P	078	>1150	5M
Y10-O	095	119	10M.
Y10-P	072	960	7M.
Y70-O	113	negl.	
Y70-P	076	152	9M.

Alcohols on galactosidase

August 8, 1949.

Lactase K-12 extract 2%. Activity ca 1200 u/ml.

.001 ml in M/50 NaP 7.5 M/2000 onpg. Stop Rx ε Na_2CO_3

Alc	Conc	20m. Rdg.
—		119
Mannitol	M/10	132
Sorbitol	M/10	133
PrOH	M/100	119
"	M/10	134 ← Optimal concentration.
"	M/1	113
"	2M	029
"	5M	006

Recheck Mannitol and PrOH concentration. Also, of 3 4/1 which showed larger alcohol effects.

8/9/49 .002 ml, as above

1	—	
2	—	
3.	PrOH	M/10
4.	Et<OH OH	M/10
5.	Mannitol	M/10
6	~~BuOH~~	~~M/10~~