

Resume of Polymerization Experiments Winter 1963 (Maleady)

In this book

Ja-9163 C^{14} PolyA: made with Brenneeman pH 6.3.
96.6 μ moles ADP, 11.3 mg plus 3.9 μ moles.

Enzyme Ja 101 G-200 1-10-63
polym ϵ ADP at least 10x stim by (pA)₃

1-14, Ja 101 cont. Primer stim ϵ UDP and ADP
apparent absolute primer dependence at
50 μ mole/lne diphosphat, 0.01 M Mg⁺⁺
Duplicated 1-30-63

1-17 Kornberg type exp't on primer formation.
Failed.

1-21-63 UDP + poly U primer. Made poly U ϵ Brenneeman pH 6.3

1-24-63 UDP + poly U. Check stimulation Brenneeman pH 6.3 enzyme.
About 2x stimulation of rate

2-1, 4-63 CDP, best at lower concentrations. (about 10 μ mole/l)
better if Mg⁺⁺ at least equal to CDP

1-7-63

ADP polymerization \bar{c}^{14} ADP (poly)

Combine 0.25 ml ADP Schwarz lot 6101, 650 mg/ml ^{250 mg total}
 and 0.1 ml \bar{c}^{14} ADP 6204 lot 68AD Schwarz

Take .01 ml and dil to 100 ml \bar{c} 1 ml INHCl & H₂O.

Abs₂₆₇ vs .01 INHCl = .427 conc = 284 μ m/ml total units 1500

	CPM	-13	CPM/ml	AV
Plate 0.25	71.1	58.1	232000	2254000
	122.4	109.4	2188000	
diluted 0.50	27.3	8.3	1660	

$\frac{22.54 \times 10^5}{284} = .793 \times 10^4$ cpm/ μ m
 7930 cpm/ μ m

Set up the following mixture and incubate at 37 °C
 after a zero time sample of .020 ml is taken & added
 to .23 ml H₂O. Take samples at 1, 2 + 3 hrs. also.

- ✓ ADP above ^{96.6} μ m .35 ml
- ✓ 5 MT in pH 9.0 .150
- ✓ .2 M MgCl₂ .100
- ✓ .004 M EDTA .15
- ✓ Enz pH 6.3 _{III} 3 .15
- ✓ (PA) 4.2 ^{2.94 mm} ml .20
- ✓ H₂O .50

Add .25 ml UrAc HClO₄ reagent
 to sample. Stand 10 min. Spin
 10 min & do a F.S. Phosphate
 on .3 ml of the sup.
 Start \approx 1:16 PM
 Freeze at 5:16 (4 inhibited) - do sewage in morning

2.10

	ADP = 96.6 μ m	2.5%	48 μ m/ml	1:1 UrAc	H ₂ O	Abs
1 Bl	-	.8	.16	.08	.3	.26
2 Std	1.1 mm/ml .080	.8	.16	.08	.3	.18
3 Zero	soln .3	.8	.16	.08	-	.26

	Abs	μ m/plate	μ m/ml	Δ	Abs	μ m/plate	μ m/ml	Δ
zero	.033	.0131	1.094		2hr	.603	.228	18.95
std	.201				std	.212		17.86

1hr	.446	.1722	14.35	13.26	3hr	.644	.237	19.76
std	.446				std	.217		18.67

4 hour total incubation (~40 μ m total)

For 20 μ moles Pi / ml = 40 μ moles total or approx 20 mg polymer

1-8-63

ADP polym. \bar{C}^{14} cont'd. (page 2)

throw mixture and do sewage. 1/4 vol $CHCl_3$ 1/10 vol isoamyl alcohol. Shake 5 min. Cent. 5 min. Take off water layer saving the $CHCl_3$ layer. Repeat \bar{c} water layer until no interface appears.

1) vol = 2 ml	2) 1.6	3) 1.4	4) 1.5	5) 1.2	6) 1.2
$CHCl_3$.5 ml	.4	.35	.375	.30	.30
alc. .2 ml	.16 .32	.14	.15	.12	.12

Combine the $CHCl_3$ layers and wash twice adding the wash to the H_2O layer. Vol = 7.1 ml

Dilute .005 to 1 ml \bar{c} H_2O and read OD vs H_2O

Abs₂₅₇ = .546 undil = 109.2/ml
total = 77.50 (or about 77.5 μ moles on basis)

Wash $CHCl_3$ layer again \bar{c} 3 ml H_2O keeping it separate and reading the OD. Abs₂₅₇ = .293 discard ^{this} 3rd washing

Soln above ~~H_2O~~ layer + 2 washings, tot vol 7.1 ml -

Dialyze vs 1 l .025 M KCl, change at 5:00 \bar{c} in morning 2 4 hrs later take out. Check OD then alc. pptr. 1-9-63

Vol = 8.2 ml Dil. .005 to 1 ml \bar{c} H_2O Abs₂₅₇ vs H_2O = .150
add 3 vols alc., 24.6 ml, .6 ml, 2 M $HgCl_2$ undil = 30 units or about 25 μ moles polymer expect 12 mg.

Wash ppt \bar{c} 95% ~~100%~~ Store in freezer over night
Label Ja 9/63

precipitate almost invisible. ~~is~~ Quite gelatinous on centrifuging

1-10/63

ADP polym. c¹⁴ cont'd (page 3)

Centrifuge 5a 9/16/63 in Antemat at 35 for 30 min. ^{Gelatinous} precipitate

Wash c 95% EtOH - 10 ml

100% EtOH - 10 ml

Ether - 5 ml. Solid didn't look too good. Very hard, small pieces. Therefore, plan to lyophilize. 1-11-63

Dissolve Poly A in H₂O. Needed .12 ml .2M versene,

Adj. pH to neutral with .030 ml 1N NH₄OH.

pH was ~5, final pH ~6.5.

Lyophilize. Weight = 11.27 mg

1-15-63

Rinse lyophilizer tube c 1/2 cc. H₂O dissolving the poly A. Store in cov. cent. tube. Dilute 0.02 ml + 0.98 ml 0.01 M KPO₄ pH 7.0. Abs₂₅₇ = 1.537 undel = 76.85. About 7.7 μmole/d = 7930 x 7.7 = ^{about} 61,100 ^{cpm}/d

Must remember that this polymer has versene in it.

Plate ^{0.01} 2 ml of solution. count in gas flow counter.

Bkgr d = 11.3

Duplicate cpm #1 558, 598 } av 593 corr to Bkgr = 582

58200 cpm #2 598, 616

58200 cpm / ml of solution

ln 7.3 μmole/d (ε_p = 10.5)

$$\frac{58,200}{7.3} = 7970 \text{ cpm} / \mu\text{mole}$$

1-10-63

G-200 Sephadex

Pool fractions 7, 8, 9 + 10 (stored in deep freeze, assayed on 1/7)

λ	Abs	Label	Ja	10163
280	0.91	mg P ₃₂ /ml =	.0792	
260	0.91			

Repeat 280/260 \bar{c} BSH in Reference cell
 280 = .099 280/260 = 1.74
 260 .057
 mg P₃₂/ml = .109

Assay

Sample	vol	2mg/ml BSA	H ₂ O	CPM	-Bg 13.4	-B ₀	Pi/plate	Pi/ml	S.A.	$\frac{S.A.}{\frac{c}{Pro} \cdot \frac{109}{ml}}$
1 Bl	-	.005	.045	28.1	14.7	-				
2 Ja 10163	.010	.005	.035	421.0	407.6	392.9	.00509	.509	6.43	4.68
3 "	.030	.005	.025	816.0	802.6	787.9	.01023	.511	6.45	4.68
4 "	.030	.005	.015	1220.0	1206.6	1191.9	.01547	.515	6.50	4.83

in each: .010 PolyA 5mg/ml
 .03% Rx Mix 1/10/63
 .010 .1M K₂HPO₄

Reassay 2-7-63

Freeze Ja 10163, fract 6, 11 + 12. Store in deep freeze in cell

New Rx Mix

- ✓ .3 ml P³² 12/5/62
- ✓ 4.0 ml .5M Tris pH 8.2
- ✓ .4 ml .05M EDTA
- ✓ .5 ml .2M MgCl₂
- ✓ .8 ml H₂O
- 6.0

Dilute .05 → 5 Plate .05, .10

	CPM	-Bg 13.4	CPM/ml
.05	1400	1386.6	27732 x 10 ⁵
.10	2730	2716.6	27166 x 10 ⁵

} 27.45 x 10⁵

$$S.A. \text{ std assay} = \frac{27.45 \times 10^5 \times 0.03}{1 + \left(\frac{45.1 \times 0.03 \times 0.3}{6} \right)}$$

1.068

S.A. = 77100 ~~cp~~

1-10-63

To test polymerization activity of myozyme ^{101 purified} Ja 9T63 from the G-200 Sephadet.

	I	II	III	IV	V
✓ ADP 1-4-63	.015	.015	.015	.015	.015
✓ .5M Tris pH 9.0	.025	.025	.025	.025	.025
✓ .2M MgCl ₂	.005	.005	.005	.005	.005
✓ .004M EDTA	.005	.005	.005	.005	.005
✓ Ja ¹⁰¹ 9T63	.010	.010	.060	.040	—
✓ (pA) _{4,2} ^{2.94mm} ml	—	.010	—	.010	—
✓ H ₂ O	.040	.030	—	—	.050

Incubate + Take samples etc. as on 1-4-63
20µl samples
Start 3:13
take sample at 10 to 5

Zero time	Phosphate dest. vol	1N H ₂ SO ₄	AmMol	Reducer	1:14 H ₂ O	H ₂ O	Abs 820	-122	µm/µl	µm/ml
1 AD		.8	.16	.08	.3	.26	.034	—		
2 50	.080	↓	↓	↓	.3	.18	.243	.209		
3 10	.3	↓	↓	↓	—	.26	.087	.053	.0203	1.69
4 20	↓	↓	↓	↓	—	.26	.069	.035	.0134	1.12
5 30	↓	↓	↓	↓	—	.26	.063	.029	.0111	.925
6 40	↓	↓	↓	↓	—	.26	.072	.038	.0145	1.21
7 50	↓	↓	↓	↓	—	.26	.075	.041	.0157	1.31

hour	Abs	-122	µm/µl	µm/ml	Δ
1	.042	—			—
2	.234	.192			—
3	.088	.046	.0191	1.59 1.69	I 0
4	.250	.218	.0907	7.66	II 6.44
5	.093	.051	.0212	1.76	III .83
6	.402	.360	.150	12.5	IV 11.29
7	.063	.021	.0087	.726	V 0

Plan regular time curve to determine actual extent of stimulation. Then Kornberg type investigation of lag period.