6/8/61

Preparation of Valyl- and Isoleucyl-RNA

	Val.	Isoleuc	Contr.	
✓ M/1 Cac. 7.0	1.5	1.5	1.5	
✓ M/10 $MgCl_2$	0.3	0.3	0.3	
✓ M/100 ATP	1.5	1.5	1.5	spec. Activity:
✓ C^{14} DL-Valine 4.2 μM/ml 5.7×10⁶ cpm/μM	0.4	—	—	1.89×10⁶ cpm/μM
✓ DL-Valine 10 μM/ml 1.52×10⁷ "	0.33	—	—	
✓ C^{14}-L-isoleucine 4.6 μM/ml	—	0.07	—	3.72×10⁶ "
✓ L-isoleucine .05 M	—	0.02	—	
✓ RNA-E 162 DU/ml	2.0	2.0	2.0	
✓ BSA 10 mg/ml	0.06	0.06	0.06	
✓ GSH .2 M	0.9	0.9	0.9	
✓ H_2O	8.0	8.6	8.7	
Val. enz 12/25	0.04		—	
IV-enz 12/25		0.07	—	
V_f	15.0	15.0	15.0	

Incubate 30'/37°. Then add 2nd batch of enzyme as indicated above, and incubate for an additional 30'. Add 1.3 gms NaCl + 30 ml cold ETOH. Ret sit 20 min in ice spin, and wash ppt. 3 × c̄ salt-ETOH. Drain well. Dissolve in 5 ml H_2O. Spin of insoluble material.

Val.:	5.05	5.10	5.00	
OD_{260} 1:100 KOH	.673	.620	.659	
OD_{260}/ml	67.3	62.0	65.9	
total OD	340	316	329	
recovery	1112			
cpm/100 μl	Δ [illegible]	.979		Nucl. autom. BG 53. Std
cpm/ml	1.11×10⁴	9.79×10³		
mμM/ml	5.90	2.64		
mμM/10 DU	.89	.42		
for 300 DU recovery	.99	.45		

6/8/61

Periodate Treatment of Valyl- and Isoleucyl-RNA.

	Val. RNA	Isoleu RNA	Control
Vol.	4.94	4.99	5.00
V M/1 Ac. pH 5.2	0.6	0.6	0.6
V M/10 Na periodate 5/7	0.6	0.6	0.6

2 hr at room Temp in the dark

Add 500 mg NaCl + 12 ml ETOH. Let sit in ice for 15'. Spin. Wash 2 x c̄ salt-ETOH. Quin. Dissolve ppt. in 5 ml cold H_2O

Nuclear automatic BG 40 Std.: 4587

cpm/50 µl	585	561
Δ	545	521
cpm/ml	1.09×10^4	1.04×10^4
mµM	5.78	2.80
mµM/10 DU	.93	.47

OD_{260} 1:100	.621	.602	.651
OD_{260}/ml	62.1	60.2	65.1
total	310	301	325
recovery			

To above solutions added 5 ml M/2 DL-Lysine buffer pH 7.0. Let sit at room Temp. 90 min. Added 850 mg NaCl + 20 ml ETOH. Let sit in ~~refrig.~~ refrig. overnight. Spun. Wash 1 x c̄ salt-ETOH. Dissolve ppt. in 5 ml M/10 Glycine buffer pH 10.11. Let sit 4 hrs at 30°. Added 0.15 ml 2 N HCl (pH 6.5-7.0) Dialyze vs 1 L H_2O c̄ 3 changes.

Vol.:	8.7	9.2	6.3
OD_{260} 1:100 KOH	.317	.314	.213
OD_{260}/ml	31.7	31.4	21.3
total OD	276 89%	288 76%	132 40%
Δ cpm/100 µl	31 31	63 76	
cpm/ml	310	630	
mµM/10 DU	.052	.054	

BG 23

6/12/61

Assay of Periodated RNA-Preps for SA-Acceptor Activity

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
✓ Lac M/1 7.0	.05	→														
✓ $MgCl_2$ M/10	.01	→														
✓ ATP M/100	.05	→														
5.7×10^6 ✓ C^{14} DL-Val. 5/26 (4.2 μM/ml)	.04	.04	.04	.04	.04											
1.5×10^7 ✓ C^{14} L-isoleuc. 4/26/59 (4.6 μM/ml)						.01	.01	.01	.01	.01	.01					
6.37×10^6 ✓ C^{14} DL-Leuc. 5/30 (6.3 μM/ml)												.03	.03	.03	.03	.03
162 DU/ml RNA-E ✓	–	.02				–	.01	.02				–	.02			
31.7 " Val sp RNA 6/12 ✓			.07						.15					.15		
31.4 " IsoLeuc sp RNA 6/12 ✓				.15						.07					.15	
21.3 Period. RNA 6/12 ✓					.25						.25					.25
BSA 2 mg/ml ✓	.01	→														
GSH. 2 M neutr ✓	.03	→														
✓ H_2O	.26	.24	.19	.11	.01	.29	.28	.27	.14	.22	.04	.27	.25	.12	.12	.12
1:50 Val. enz. ✓	.05	.05	.05	.05	.05											
1:25 ~~Leuc~~ IV-enz ✓						.05	.05	.05	.05	.05	.05					
1:10 Leuc enz. ✓												.05	.05	.05	.05	.05

dil. in M/50 KPO_4 7.5+ M/20 2-MSH

30'/37°

cpm/0.5	26	493	483	102	57	63	496	974	136	502	106	63	905	111	116	61
Δ		967	457	76	21		433	911	73	439	43		842	48	53	0
cpm/ml		1934	914	154	42		866	1822	146	878	86		1684	96	106	
mμM		.340	.160	.027	.007		.058	.121	.0097	.059	.006		.264	.015	.017	
mμM/10 DU		1.05	.72	.052	.014		.36	.38	.02	.27	.011		.82	.032	.035	

.37