

Renilla Luciferase Synthesis

Reagents	Volume (μl)
S-100	4
55 mM Met	2
100 mM MgCl ₂	4
Synthesis mix	16
1 μg/μl Plasmid pRL-nullR9 (Bam HI digested)	1
10 μg/μl Total tRNA	6
T7 RNA polymerase	3
50 μM Coelentarazine	4
	40 μl
70S	2 pmol
S-30 buffer	To 30 μl
Total volume	70 μl

Prepare ribosome:

30s and 50s subunits were heat activated in S-30 buffer for 20min at 42°C and then associated in the same buffer for 10 min at 37°C.

Test program Genios (Tecan):

37°C, 80 cycles (Signal will reach the max around 2h, 60cycles), 2min interval, 5000 ms integration time, 150 Gain

MATERIALS

Synthesis mix: Synthesis mix (below table) is prepared and stored at -80C in 50 μl aliquots.

Reagents	Volume (μl)	Sigma Catalog #
2 M HEPES-KOH, pH 8.2	200	H-4034
0.55 M Dithiothreitol	25	D-9779
38 mM ATP, pH 7.0 (KOH)	250	di-Na ⁺ salt, A2383
CTP, GTP, UTP mixture [each at 88 mM, pH 7.0 (KOH)]	75	CTP (Na ⁺) C-1631 GTP (Na ⁺) G-8877 UTP (Na ⁺) U-6625
0.42 M Phosphoenolpyruvate (adjust pH 7.0 with acetic acid)	500	tri-Na ⁺ salt, P-7002
19 Amino acids, 55 mM each (minus methionine & cysteine)	50	LAA-21
40%(w/v) PEG-8000 in water	375	
2.7 mg/ml Folinic acid in water	100	Ca ²⁺ salt, F-7878
2000 U/ml Pyruvate kinase in 20% glycerol	240	P-9136
4.2 M Ammonium acetate	66.5	
8.4 M Potassium acetate	66.5	
Total volume	1946	

S-100: Prepared as described in separate procedure online.

Coelentarazine (FW: 423.5)

Purchased 250 µg Coelentarazine from Promega cat# S2001A. Resuspend 250 µg in 500 µl of 200 proof ethanol to get 1 mM stock solution. Store at -80C.

Dilute stock 1:20 with S-100 buffer to get the 50 µM working stock solution.

Folinic Acid (FW: 511.5)

Dissolve 27 mg in 10 ml of water to get 2.7 mg/ml stock.

Phosphoenol pyruvate (FW: 234)

Dissolve 234 mg in 2.4 ml water to get 0.42 M stock. Adjust pH 7.0 with acetic acid (or with 2M Tris base if pH is low).

Pyruvate kinase

Dissolve 5000 Units in 2.5 ml of 20% glycerol to get 2000 U/ml stock

Potassium acetate (FW 98.14)

Dissolve 8.3 Grams in 10 ml for 8.4 M stock

DTT (FW: 154.25)

Dissolve 85 mg in 1 ml for 0.55 M stock

HEPES-KOH, pH 8.2 (FW: 238.3)

Dissolve 14.4 Grams in 30 ml for 2 M stock (adjust pH before making final volume).

CTP, UTP, GTP Mix (88 mM each)

Dissolve 61 mg CTP + 59 mg UTP + 61 mg GTP in 1140 µl of water (final volume). Check pH and adjust to 7.0 with KOH. The final conc. is 88 mM for each.

Total tRNA from E. coli strain W, Type XX (Sigma cat# R-1753)

Dissolve 500 Units in 2 ml water to get final conc of 10 µg/µl stock. Take OD at 260 nm to determine the conc.

38 mM ATP

Add 192.3 µl of 100 mM ATP to 307.7 µl water to get 38 mM stock.

4.2 M Ammonium acetate

Add 840 µl of 5M ammonium acetate to 160 µl of water to get 4.2 M stock.

Store everything as small aliquots in -80C. Try not to run out of more than 1 reagent at the same time. Keep backup stocks until you are sure that the new stock reagent is working.

PLATE, 96 wells, White, VWR Cat# 82050-726 case of 40 for \$42.85

55 mM Methionine (FW: 149)

Dissolve 75 mg in 10 ml water.

19 Amino acid mix (55 mM each)

Alanine (FW: 89)	49 mg
Arginine (FW: 210.7)	110 mg
Asparagine (FW: 132)	70 mg
Aspartic acid (FW: 133)	70 mg
Cysteine (FW: 121)	65 mg
Glutamine (FW: 146)	75 mg
Glutamic acid (FW: 147)	75 mg
Glycine (FW: 75)	40 mg
Histidine (FW: 209.6)	110 mg
Isoleucine (FW: 131)	67 mg
Leucine (FW: 131)	67 mg
Lysine (FW: 182.6)	93 mg
Phenylalanine (FW: 165)	90 mg
Proline (FW: 115)	60 mg
Serine (FW: 105)	54 mg
Threonine (FW: 119)	60 mg
Tryptophan (FW: 204)	110 mg
Tyrosine (FW: 181)	90 mg
Valine (FW: 117)	60 mg
Water (Final volume)	10 ml