

3.7 Recipes

E mix

0.1M ATP	10 uL
0.1M GTP	10 uL
0.4M CP	25 uL
19 aa -met	40 uL
35S Met (250 uCi)	100 uL
2M Tris base	3 uL or as needed to pH 7
H ₂ O	12 uL
Total	200

CB 20x

	10% linked	unlinked
4M KAc	5 mL	5 mL
2M Tris Ac pH 7.5	1 mL	1 mL
1M MgCl ₂	0.2 mL	0.5 mL
1M DTT	-	0.2 mL
H ₂ O	3.8 mL	3.3 mL
Total	10.0 mL	10.0 mL

tRNA 10 mg/mL PIN 200X

10mg/mL chymostatin (DMSO)	20 uL
10mg/mL antipain (DMSO)	20 uL
10mg/mL leupeptin (H ₂ O)	20 uL

10mg/ml pepstatin (H ₂ O)	20 uL
2mg/mL aprotinin (H ₂ O)	200 uL
Water	9.72 mL
Total	10.0 mL

Translation Reaction 10 uL, 10% linked

CB 20X	0.5 uL
E mix	2.0
RRL	4.2
tRNA 10mg/mL	0.1
PIN 200X	0.1
CK	0.1
RNasin	0.1
Water	1.4
	8.5 uL
SP6 transcripts	1.0
membranes	0.5
TOTAL	10.0 uL

Incubate 24°C 1 h. Transfer onto ice to stop reaction. 1 uL of this reaction run on SDS-PAGE and fluorographed is usually sufficient to visualize with an overnight exposure.