

4.7 Recipes

CB 10X

- For 20% WG:

	CB 10X unlinked	CB 10X 10% linked	Final in reaction
1M Hepes-KOH pH 7.5	120 uL	40 uL	20 mM
4M KOAc	300	300	140 mM
1M MgOAc ₂	10	-	2.4 mM
0.1M spermidine	40	20	0.4mM
1M DTT	12	2	2 mM
0.5 M EDTA	2	2	0.1 mM
Water	516	636	
Total	1 mL	1 mL	

E mix, tRNA, pin

- same as for RRL

Translation reaction 10 æL, 20% WG, 10% linked

CB 10X	1 uL
E mix	2
WG	2
tRNA 10mg/mL	0.1
PIN	0.1
RNasin	0.1
CK	0.1

Water	3.1
	8.5 uL
Transcripts	1
Membranes	0.5
Total	10 uL

Incubate 24°C 1 h. Transfer onto ice to stop reaction. 1 uL of this reaction run on SDS-PAGE and fluorographed can usually be visualized after an overnight exposure.