4.4 Buffers and reagents

A. CB 10X

Final desired concentrations are 20 mM HEPES, 2.5 mM Mg, 0.4 mM spermidine, 140 mm KAc, and 2 mM DTT.

WG is in buffer of 40 mM HEPES, 100 mM K, 5 mM Mg, 4 mM DTT.

Contributions from transcription using 10% of translation volume as transcript are 8 mM HEPES (pH 7.5), 1.4 mM Mg, 0.2 mM spd, 1 mM DTT. (Note: If you need to increase the % of transcript, you will have to consider the high Mg-concentration (14 mM) in the transcription reaction.)

For optimizing WG amounts from 10% to 40%, a different CB10X must be made for each reaction:

WG CB10X unlinked (volumes in uL)

	10%	**15%**	**20%**	**25%**	**30%**	**35%**	**40%**
1M Hepes pH 7.6	160	140	120	100	80	60	40
4M KOAc	325	312	300	287	275	262	250
1M MgOAc2	20	17.5	15	12.5	10	7.5	5
0.1M spermidine	40	40	40	40	40	40	40
1M DTT	16	14	12	10	8	6	4
0.5M EDTA	2	2	2	2	2	2	2
Water	437	474	511	548	585	622	659
TOTAL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL	1 mL

WG CB10X 10% linked (volumes in uL)

	10%	**15%**	**20%**	**25%**
1M Hepes pH 7.6	80	60	40	20
4M KOAc	325	312	300	287

1M MgOAc2	5	2.5	-	-
0.1M spermidine	20	20	20	20
1M DTT	6	4	2	-
0.5M EDTA	2	2	2	2
Water	562	599	636	670
Total	1 mL	1 mL	1 mL	1 mL

B. E mix - same as for RRL.

C. WG - desalted, nuclease-treated. Current batches (Nov.'93) are typically used at 20% final concentration.

D. tRNA, CK, RNasin, pin - as for RRL.