PREPARATION OF HIGH SPEED EXTRACT

1XMMR (salts per 10liters):

100mM NaCl	58.4g NaCl
2mM KCl	1.49g KCl
1mM MgSO4	2.46g MgSO4X7H2O
2mM CaCl2	2.94g CaCl2
5mM HEPES	11.9g HEPES
0.1mM EDTA	0.675g EDTA

200mls:

20X XB salt stock	2M KCl	29.8g solid
	(74.56g/mole)(2mole/l)(0.2l)	
	20mM $MgCl_2$	4mls 1M; 1:50
	2 mM $CaCl_2$	0.058 g $CaCl_2$. $2H_2O$
		or 0.4 mls of 1M sk.

200 mls XB		[final]
10ml	20X XB salt stock	100mM KCl
		0.1mM $CaCl_2$
		1 mM $MgCl_2$
2ml	HEPES 1M stock (pH 7.7 at 15 mM with KOH); 1:100 dilution	10mM
6.66ml	sucrose 1.5M stock; 1:30 dilution	50mM

300mls 2% cysteine in 1X XB salts, pH 7.8

50 mls CSF-XB

2.5 mls	20X XB salt stock
50 μl	1M $MgCl_2$
500μl	1M HEPES stock
1.665ml	1.5M sucrose stock
500μl	0.5 M EGTA (1:100)
44.8 mls	H_2O

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plus, for some steps, dilute protease inhibitors 1:1000

leupeptin (10mg/ml combined stocks in DMSO)
chymostatin
pepstatin

1M $CaCl_2$
Versilube

Energy Mix (aliquots at -20°C):

150 mM creatine phosphate (BMB; 127574)	For 15ml=0.736g
20 mM ATP (BMB 519979)	0.181g

20 mM $MgCl_2$ (from 1M stock; 1:500)
store in aliquots at -20°C