WEB272

Versuchsplanung SOMT refolding screen

Factors

- **Arginine**
- **pH**
- **divalent cation (Mg^{2+}, Ca^{2+})**
- **ionic strength (NaCl, KCl)**
- **glycerin**
- **Redox system**
- **Cyclodextrin**
- **SAH**
- temperature

Faktor/Parameter	*Symbol*	*Setting*		*Unit*
		-1	**+1**	
pH	A	5.5	9.5	-
Arginine	B	0	0.5	M
Glycerin	C	0	10	%
div. Cation	D	1 mM EDTA	2 mM $CaCl_2$, $MgCl_2$	mM
Ionic strength (NaCl/KCl, each)	E	Low (10 mM NaCl, 0.5 mM KCl)	High (250 mM NaCl, 10 mM KCl)	mM
Redox	F	5 mM DTT	1 mM GSH/ 0.2 mM GSSG	mM
Cyclodex	G	0	0.03	M
SAH	H	0	0.5	mM
Buffer	-	50 mM Mes / Borate		
Temperature	-	22°C (RT)		
Time	-	4h		

Stocks to prepare:

- 0.1 M Mes pH 5.5 **[A1B1]** ✓
- 0.1 M Mes, 1 M Arginine pH 5.5 **[A1B2]** ~~✓~~

- 0.1 M Borate pH 9.5 **[A2B1]** ✓
- 0.1 M Borate, 1 M Arginine pH 9.5 **[A2B2]** ✓

- 200 mM $MgCl_2$, 200 mM $CaCl_2$ **[C1]** ✓ →
- 0.5 M EDTA **[C2]** ✓

- 2.5 M NaCl, 100 mM KCl **[D1]** ✓
- 0.1 M NaCl, 5 mM KCl **[D2]** ✓

- 1M DTT **[E1]** ✓
- 50 mM GSH, 10 mM GSSG **[E2]** →

- 5 mM SAH **[F1]**
- Glycerin **[G]** ✓
- 120 mM a-Cyclodex **[H]** ✓

Arg 210.67

MeS 195.24

$MgCl_2 \times 6H_2O$ 203.31

$CaCl_2$ 110.99

Borat 61.83

50 ml

10.53 g Arg / 0.976 g Mes

10.53 g Arg / 0.303 g Borate

10 ml: 0,407 g $MgCl_2 \times 6H_2O$, 0.220 g $CaCl_2$

100 µl GSH, 20 µl GSSG, 80 µl H_2O

Prec.

Buffers to prepare:

Prec.	No.	Buffer (1 mL each)	Add: (µl) A1B1	A1B2	A2B1	A2B2	C1	C2	D1	D2	E1	E2	F	G	H	H_2O
–	1	pH 9.5 0.5 M Arg 10% Glycerin low ionic EDTA DTT SAH	-	-	-	500	-	2	-	10	5	-	100	100	-	283
–	2	pH 5.5 low ionic EDTA DTT	500	-	-	-	-	2	-	10	5	-	-	-	-	483
+	3	pH 9.5 10% Glycerin Low ionic MgCa GSH:GSSG CycloDex	-	-	500	-	10	-	-	10	-	20	-	100	250	110
0	4	pH 5.5 0.5 M Arg 10% Glycerin High ionic EDTA GSH:GSSG Cyclodex	-	500	-	-	-	2	10	-	-	20	-	100	250	118
–	5	pH 9.5 0.5 M Arg High ionic EDTA GSH:GSSG	-	-	-	500	-	2	10	-	-	20	-	-	-	468
+	6	pH 5.5 0.5 M Arg High ionic MgCa DTT Cyclodex SAH	-	500	-	-	10	-	10	-	5	-	100	-	250	125
+	7	pH 9.5 0.5 M Arg MgCa DTT Cyclodex	-	-	-	500	10	-	-	10	5	-	-	-	250	225
+	8	pH 5.5 10% Glycerin High ionic EDTA DTT Cyclodex SAH	500	-	-	-	-	2	10	-	5	-	100	100	250	33
–	9	pH 9.5 10% Glycerin High ionic MgCa DTT	-	-	500	-	10	-	10	-	5	-	-	100	-	375
+	10	pH 5.5 EDTA GSH:GSSG Cyclodex SAH	500	-	-	-	-	2	-	10	-	20	100	-	250	118
–	11	pH 9.5 High ionic MgCa GSH:GSSG SAH	-	-	500	-	10	-	10	-	-	20	100	-	-	360
–	12	pH 5.5 0.5 M Arg 10% Glycerin MgCa GSH:GSSG SAH	-	500	-	-	10	-	-	10	-	20	100	100	-	260

1ml + 50µl 1mg/ml SOX4

- no prec
+ heavy prec
o medium prec

* X – A → 100 µl abgenommen + 20 µl TCA

X / → rückständiges Pellet

	tara (g)	eluted (g)	recovery (mg)
1	1.152	1.206	
2	1.151	1.191	
3	1.147	1.184	
4	1.148	1.193	
5	1.15	1.188	
6	1.148	1.193	
7	1.157	1.195	
8	1.145	1.181	
9	1.133	1.173	
10	1.142	1.180	
11	1.147	1.183	
12	1.145	1.207	