S.pombe – Media Recipes

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NOTE 1: Tables supersede those in the Keogh Lab general media list

NOTE 2: Autoclave 121°C 20 - 30 min to sterilize

NOTE 3: Use *Bacto Yeast Extract* or *Bacto Agar* from **BD Biosciences** (Sp shows defective growth in media from alternate suppliers).

YES (Rich media) **(*YE :** w/o adenine**)**

	100ml	200ml	300ml	500ml	1L
Glucose (g)	3	6	9	15	30
Yeast Extract (g)	0.5	1	1.5	2.5	5
ade/his/lys/ura (g, ea)	0.0225	0.045	0.0675	0.1125	0.225
Leucine (g)	0.025	0.05	0.075	0.125	0.25
Agar (g)	2	4	6	10	20

EMM (minimal media w/o **thiamine**)

	100ml	200ml	300ml	500ml	1L
Glucose (g)	2	4	6	10	20
KH phthalate (g)	0.3	0.6	0.9	1.5	3
Na_2HPO_4 (dibasic, g)	0.22	0.44	0.66	1.1	2.2
NH_4Cl (g)	0.5	1	1.5	2.5	5
ade/his/lys/ura (g, ea)	0.0225	0.045	0.0675	0.1125	0.225
Leucine (g)	0.025	0.05	0.075	0.125	0.25
Agar (g)	2	4	6	10	20
50X salts (ml)	2ml	4ml	6ml	10ml	20
1000X vitamins (µl)	100ul	200ul	300ul	500ul	1000
5000X minerals (µl)	20ul	40ul	60ul	100ul	200
ddH_2O (ml)	98ml	186ml	294ml	490ml	980

NOTES:

- If you want to see the red color associated with *ade6* mutations reduce adenine to 10mg/L
- Use EMM to activate the expression of pREP series vectors : these contain the repressible nmt1 promoter (***n***o ***m***essage in ***t***hiamine)
- Expression of pREP series: pREP1/2 (strong), pREP41/42 (medium), pREP81/82 (weak)
    1. Grow cells in EMM (w/ thiamine) in exponential phase
    2. Harvest cells (3K rpm, 3min) and wash cells 2-3 times with EMM (NO thiamine)
    3. Resuspend cells in EMM (w/o thiamine). Grow cells >12 hrs (12 – 17 hrs, optimal is gene dependent)
    4. Harvest cells

SD (*S. cerevisiae* minimal media w/ **Thiamine**)

	100ml	200ml	300ml	500ml	1L
Glucose (g)	2	4	6	10	20
YENB w/o a.a (g)	0.67	1.34	2.01	3.35	6.7
ade/his/lys/ura (g, ea)	0.0225	0.045	0.0675	0.1125	0.225
Leucine (g)	0.025	0.05	0.075	0.125	0.25
Agar (g)	2	4	6	10	20

NOTES:
- If you want to see the red color associated with *ade6* mutations reduce the adenine to 10mg/L
- Because SD media contains thiamine, it can't be used for the overexpression of pREP vectors

SPA (Sporulation media for rapid conjugation and mating)

	100ml	200ml	300ml	500ml	1L
Glucose (g)	3	6	9	15	30
KH_2PO_4 (monobasic, g)	0.5	1	1.5	2.5	5
1000X Vitamins (ml)	0.1ml	0.2ml	0.3ml	0.5ml	1ml
ade/his/lys/ura/leu * (g, ea)	0.0045	0.009	0.0135	0.0225	0.045g
Agar (g)	3	6	9	15	30g

NOTES:
- * 1/5 amount of usual supplements
- **Mating**
  1. Mix same amount of fresh cells from media (OD_{600}0.5 / 5ml) or plate. Wash 2X with 1ml ddH_2O.
  2. Resuspend cells in 20ul DW and spot on SPA plate. Incubate plate at RT for 4 days.

Media additives

G418 (geneticin sulfate, neomycin sulfate; eg. American Bioanalytical, AB05057): Use at 100μg/ml final (0.2μm filter sterilized 50mg/ml stock in ddH_2O at –20°C). Plates are stable for >6 months at 4°C. **G418 does not work in minimal EMM media** [though it works well in PMG medium, which replaces the NH_4Cl (5g/L) with L-Glutamic acid, monosodium salt (a.g. Sigma G-5889)].

Noursethricin (clonNAT, Nat; Werner Bioagents): Use at 100μg/ml final (0.2μm filter sterilized 100mg/ml stock in ddH_2O at –20°C). Plates are stable for >6 months at 4°C.

NOTE 4: When transforming KanMX and NatMX cassettes into *S. pombe*, it is strongly advised to replica plate 18 - 20hrs after transformation. If omitted, high background makes it difficult to identify true $G418^R$ or Nat^R colonies (Goldstein & McCusker (1999) *Yeast* **15:**1541).