Media Additives

Antibiotics, counterselection agents & inhibitors

NOTE 1: Use in conjunction with the **Media Recipes** protocol.

NOTE 2: NEVER autoclave media with an additive. Generally an additive should not be added to agar-based media until cooled to ≈ 50°C (just about cool enough to hold in your palm). Mix well (solubility is an issue with some of these things), 70% EtOH spritz and flame to remove bubbles (**careful**) and pour plates (generally a little thinner than normal to keep costs down).

Antibiotics (*E.coli*)

Ampicillin: (Amp) 1000 x stock = 50mg/ml in ddH_2O (>99% of the time – some older plasmids have special requirements: **stringency issue**). 0.2μ filter sterilize and store 1ml aliquots at –20°C. Plates stable for >6 months at 4°C. Mixed media can be used after a week or two at RT°C (but storage at 4°C is recommended).

NOTE: Amp is bacteristatic rather than bactericidal. β-lactamase (the Amp^R product) is secreted into the media around Amp^R colonies, so satellites can develop if the plates are incubated for too long.

Kanamycin: (Kan) 1000 x stock = 50mg/ml in ddH_2O (>99% of the time – some older plasmids have special requirements: **stringency issue**). 0.2μ filter sterilize and store 1ml aliquots at –20°C. Plates stable for >6 months at 4°C. Mixed media can be used after a week or two at RT°C (but storage at 4°C is recommended).

NOTE: Kan is bactericidal – satellites not a problem

Chloramphenicol: (Chl) 1000 x stock = 34mg/ml in EtOH. Store at –20°C. Usually used to drive-up the yield of some low-copy plasmids: rarely done nowadays.

Tetracycline: (Tet) 1000 x stock = 5mg/ml in EtOH. Store at –20°C. Usually used to maintain the F' plasmid of some color-selectable strains (check the genotype maps) prior to making competent cells.

Antibiotics (*S.cerevisiae* / *S.pombe*)

G418: (G418, geneticin sulfate, neomycin sulfate; eg. American Bioanalytical AB05057) 250 x stock = 50mg/ml in ddH_2O. 0.2μ filter sterilize and store 1ml aliquots at –20°C for lab stocks. Plates (usually @ 200μg/ml in YPD) stable for >6 months at 4°C.

NOTE: When transforming the KanMX cassette into yeast it is strongly advised to replica plate 24hrs after transformation. If this is omitted there is usually a high background and it can be difficult to identify true $G418^R$ colonies.

Noursethricin: (clonNAT, Nat) 1000 x stock = 100mg/ml in ddH_2O. 0.2µ filter sterilize and store 1ml aliquots at –20ºC. Plates (usually made in YPD) stable for >6 months at 4ºC.

NOTE: (from Werner Bioagents, http://www.webioage.com/seite5.html) ClonNAT, trade name for nourseothricin (a complex of the streptothricins F and D), produced by *Streptomyces noursei*, as a dihydrogen sulphate. Inhibits bacteria, mycobacteria, mycoplasms, yeasts, viruses and plants by inhibiting ribosomal protein synthesis and inducing miscoding.

Other additives (*S.cerevisiae*)

α-aminoadipic acid: (DL-αAAA; eg. US Biological A1374-09 5g $104) Use at 0.2% final. Used to counterselect *LYS2*$^+$.
Stock = 2g / 50ml (4%) in ddH_2O. Adjust pH to 6.0 with 5M KOH, 0.2µ filter sterilize and store at 4ºC. Use 50ml of this per L media (0.2% final): **see specific protocol on LYS Counterselection (http://mckeogh.googlepages.com)**
NB. Must add LYS$^+$ to media (3.3ml 100mM stock / L)

NOTE: Solubility is an issue and the pH-ing to 6.0 step is absolutely required to get the αAAA into solution. It may help to initially overshoot (to ≈ 8.0) and as more αAAA dissolves come closer to the desired 6.0. This will take hours: plan accordingly. 5-FOA is more commonly used as a counterselection agent

CLASS: Counterselection agent

5-FOA: (5-FluorO-Orotic acid; eg. US Biological F5050 10g $199) Use at 1mg/ml. Used to counterselect *URA3*$^+$. **Use URA$^+$ synthetic complete plates** (the conversion of 5-FOA to the toxic metabolite requires a functional *URA3*, and you're selecting against this) to shuffle out a *URA* plasmid.

NOTE: Solubility is an issue. Add appropriate amount for final volume to 2 x synthetic complete medium (+ URA). Place on shaker for an hour or two at 37ºC. Add an equal volume of >70ºC 2 x Agar (40g/L in ddH_2O), Mix well, 70% EtOH spritz and flame to remove bubbles (**careful**), and pour plates (generally a little thinner than normal to keep costs down). Plates stable for >6 months at 4ºC.

CLASS: Counterselection agent

6AU: (6-AzaUracil) Stock = 100mg/ml in DMSO. Store at –20ºC.
Commonly used at 75µg/ml final in URA$^-$ synthetic complete plates. Plates stable for >6 months at 4ºC.
Transcription elongation inhibitor; works by depleting nucleotide pools (see also MPA). The massive amount of Uracil in URA$^+$ plates kills this. Strain should be URA$^+$ (ie. *URA3* in the genomic locus, or transformed with a cen/ars *URA3* plasmid, eg. pRS316).

NOTE: While commonly used at 75µg/ml, it is recommended to test a variety of concentrations: 0, 10, 25, 50, 75 and 200 (all µg/ml final). *ppr2*Δ (TFIIS) shows a

sensitivity at 10, although most don't show problems until > 25 (Keogh *et al* (2004) *Mol Cell Biol* **23:**7005).
CLASS: Transcription elongation inhibitor

Benomyl: (Ben) Commonly used 15μg/ml final in YPD plates. **Horribly insoluble**.
Used in: Krogan *et al* (2004) *PNAS* **101:**15313; Keogh *et al* (2006) *Genes Dev* **20:**660.
CLASS: Microtubule destabilizer, chromosome stability analyses.

Bleomycin: (bleo) Commonly used at 5μg/ml final in YPD plates. **Expensive**. Generates free radicals and induces DNA lesions similar to those caused by ionizing radiation.
Used in: Keogh *et al* (2006) *Nature* **439:**497.
CLASS: DNA damaging agent / genotoxin

Camptothecin: (CPT) Commonly used at 20μM final (7mg/L, 100mg bottle) in YPD plates. Topoisomerase inhibitor that induces DSBs by causing replication forks to stall.
CLASS: DNA damaging agent / genotoxin

HydroxyUrea: (HU) Use at 100mM final (Yes, that is milliMolar), commonly in YPD medium or plates. Inhibitor of dNTP synthesis, leads to DNA replication fork collapse. Also used to induce a cell-cycle arrest (early S-phase).
Used in: Keogh *et al* (2006) *Nature* **439:**497; Keogh *et al* (2006) *Genes Dev* **20:**660.
CLASS: DNA damaging agent / genotoxin, cell-cycle arrest

MethaneMethyl Sulfonate: (MMS) 100% stock in chemical cabinet. Use at 0.05 - 0.1% final (commonly in YPD medium or plates). Alkylating agent.
Used in: Keogh *et al* (2006) *Nature* **439:**497; Keogh *et al* (2006) *Genes Dev* **20:**660.
CLASS: DNA damaging agent / genotoxin

MycoPhenolic acid: (MPA) Commonly used at 15μg/ml final in URA^- synthetic complete plates. Transcription elongation inhibitor; works by depleting nucleotide pools (see also 6AU). The massive amount of Uracil in URA^+ plates kills this. Strain should be URA^+ (ie. *URA3* in the genomic locus, or transformed with a cen/ars *URA3* plasmid, eg. pRS316).
NOTE: When working with a possible transcription elongation factor, it is recommended to test for MPA sensitivity (it usually mirrors 6AU), but the latter is more commonly used for in-depth experiments (Keogh *et al* (2004) *Mol Cell Biol* **23:**7005).
CLASS: Transcription elongation inhibitor

Nocodazole**:** (noc) Make a 1.5mg/ml stock in DMSO; this is 100x. (Sigma M1404 >99%TLC; 10mg $54, 50mg $215). Destabilizes microtubules preventing sister chromatin separation: G_2/M phase.

NOTE: Nocodazole escape can occur (particularly with checkpoint mutants). Arresting cells for too long is eventually lethal. Nocodazole arrest is strain dependent (too much can induce break-through, results are also problematic at 37°C).
Used in: Keogh *et al* (2006) *Genes Dev* **20:**660.
CLASS: Microtubule destabilizer, cell-cycle arrest

Sodium Butyrate: Histone DeACetylase (HDAC) inhibitor. Use at 5 – 50mM final (test at 0.5mM). Relatively cheap, but nowhere near the potency of Trichostain A (TsA).
CLASS: HDAC inhibitor

Trichostain A: (TsA) HDAC inhibitor (see also Sodium Butyrate). Use at 30nM – 3μM final. **Very Expensive**. Used in (Keogh *et al* (2005) *Cell* **123:**593).
CLASS: HDAC inhibitor