# Protocol for Sysbio plates and media

| **LB plates:** | **1 L** | **4 L** |
|---|---|---|
| Peptone from casein (Tryptone) | 10 g | 40 g |
| NaCl | 10 g | 40 g |
| Yeast Extract | 5 g | 20 g |
| Set pH to 7.0 with NaOH | | |
| Agar agar | 16 g | 64 g |
| Autoclave | | |

**LB + Amp 100 mg/L plates:**
**Follow protocol for LB plates, add ampicillin after autoclavation.**
Ampicillin sodium salt: 100 mg/ml (1000X stock) dissolved in 50% EtOH/$H_2O$ and sterile-filtered.
*OBS! Handle ampicillin with care and avoid breathing in dust/fumes.*
**LB + Chl 25 mg/L plates:**
***Chloramphenicol:*** 25 mg/mL (1000x stock) dissolved in 100% EtOH and sterile filtered (store at -20°C). Final concentration to use 25 mg/L (=1mL/L) for both media and plates. Add after autoclavation.
**Make stock:**
Weigh 0.25 g of Chloramphenicol. Add 10 ml of 95% EtOH (70% may also be used), dissolve completely. Stock may be kept at -20°C for 1 year.

| **YPD plates:** | **1 L** | **4 L** |
|---|---|---|
| Peptone from meat | 20 g | 80 g |
| Yeast Extract | 10 g | 40 g |
| Agar agar | 20 g | 80 g |
| H2O | 0,8 L | 3,5 L |
| Autoclave | | |
| D(+)-Glucose | 20 g | 80 g |
| H2O | 0,2 L | 0,5 L |
| Autoclave glucose separately | | |

**YPD + G418 200 mg/L plates:**
***Follow protocol for YPD plates, add G418 after autoclavation.***
***G418 disulfate salt:*** 50mg/ml dissolved in $H_2O$ and sterile-filtered (or Formedium G418-S solution 50 mg/ml sterile-filtered). Final concentration: 200 mg/L (=4ml/L).
*OBS! Handle G418 with care and avoid breathing in dust/fumes.*

**YPD + G418 200 mg/L + nourseothricin 100 mg/L plates:**
***Follow protocol for YPD plates, add G418 and NAT after autoclavation, when temperature of agar mix is 50°C.***
*Add 4mL G418 50mg/ml. Final concentration: 200 mg/L (=4ml/L).*
*Add 1 mL nourseothricin 100 mg/uL.*

| **SD dropout plates:** | **1 L** | **4 L** |
|---|---|---|
| *Solution 1:* | | |
| Agar agar (final conc. 2%) | 20 g | 80 g |
| H2O | 750 ml | 3 L |
| Autoclave and keep at a room temperature of 60°C. | | |

*Solution 2:*

| | | |
|---|---|---|
| Complete supplement mix Drop out (select one of the below dropout aa mix) | | |
| -URA | *0.77 g* | 3,08 g |
| -HIS | *0.77 g* | 3,08 g |
| -URA -HIS | *0.75 g* | 3 g |
| Yeast Nitrogen Base without amino acids | 6.9 g | 27,6 g |
| D(+)-Glucose | 20 g | 80 g |
| $H_2O$ | 250 ml | 1 L |
| Set pH to 5.5 – 6.0 | | |

Let the solution dissolve and set pH. Gently heat Solution 2 in the microwave until the temperature is about 60°C. Stir until completely dissolved and filter-sterilize. Add solution 2 to the autoclaved agar-solution (solution 1) and mix thoroughly.

| **5-FOA plates:** | **600ml** | **4 L** |
|---|---|---|
| *Solution 1:* | | |
| Agar agar (final conc. 2%) | 12 g | 80 g |
| $H_2O$ | 255 ml | 3 litre |
| Autoclave and keep at a temperature of 60°C. | | |
| | | |
| *Solution 2:* | | |
| Yeast Nitrogen Base without amino acids | 4.14 g | 27,6 g |
| Complete supplement mix Drop out: -URA | 462 mg | 3,08 g |
| Uracil 99+% | 30 mg | 0,2 g |
| 5-Fluoroorotic acid | 600 mg | 4 g |
| D(+)-Glucose | 12 g | 80 g |
| MQ $H_2O$ | 345 ml | 1 litre |

Gently heat Solution 2 in the microwave until the temperature is about 60°C. Stir until dissolved completely. Filter-sterilize. Add the autoclaved agar-solution (solution 1). Mix thoroughly, pH is not set.

*OBS! Handle 5-FOA with care and avoid breathing in dust/fumes.*

| **Chemicals used:** | **Brand** | **Product number:** |
|---|---|---|
| Peptone from casein | Merck | art nr 1.07213.1000 |
| NaCl | Merck | art nr 1.06404.1000 |
| Yeast Extract | Merck | art nr 1.11926.1000 |
| Agar agar | Merck | art nr 1.01614.1000 |
| Ampicillin sodium salt | AppliChem | art nr A0839.0025 |
| Peptone from meat | Merck | art nr 1.07224.1000 |
| D(+)-glucose | Merck | art nr 1.08342.9025 |
| G-418 disulfate salt | Sigma Aldrich | art nr A1720 |
| Complete supplement mix Drop out: -URA | Formedium | art nr DCS0169 |
| Complete supplement mix Drop out: -HIS | Formedium | art nr DCS0079 |
| Complete supplement mix Drop out: -HIS,-URA | Formedium | art nr DCS0529 |
| Yeast Nitrogen Base without amino acids | Formedium | art nr CYN0402 |
| Uracil 99+% | Alfa Aesar | art nr A15570 |
| 5-Fluoroorotic acid | Formedium | art nr 5FOA10 |

Document updates:

| **Name** | **Date** | **Update** |
|---|---|---|
| Åsa Rensfeldt | 2016-04-04 | Addition of amounts for 4 L batches and safety reminders regarding antibiotics and 5-FOA. |
| Angelica Ardehed | 2016-11-18 | New article no and company for 5-FOA |
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