Preparation of holidic medium for *Drosophila*

Reference: (Piper et al., 2014)

Methods website: <http://www.nature.com/protocolexchange/protocols/2829>

# Introduction

*Drosophila* *melanogaster* feeds on fermenting fruit, and experiences relatively high concentrations of ethanol and organic acids (low pH). Thus, a simple diet of sucrose and lyophilized yeast, with a weak organic acid as preservative, is a sufficient diet.

This recipe describes a fully defined synthetic (holidic) medium that it is suitable for adults and sufficient for fruitfly development, albeit at a reduced rate. When adult flies are maintained on the diet, they are phenotypically similar (fecundity & lifespan) to those kept on a natural yeast based (oligidic) diet. The holidic medium can produce more stable experimental outcomes than oligidic media, thus potentially improving inter-laboratory comparability, and greatly improved oral drug delivery to flies. This medium offers the opportunity to investigate the effects of subtle nutrient manipulations that could not be achieved otherwise.

Approx. cost for media alone: ~£16 per litre (2012 prices)

References to original work describing optimal ranges for each nutritional component: (Sang, 1956)

Reviewed in: (Sang, 1978)

Modified in: (Sparrow and Sang, 1975)

# Procedure

## IN ADVANCE

1. purchase all ingredients according to tables attached (Table 1, p.3 & Table 2, p.4)
2. prepare the stock solutions using the quantities and according to the directions in Stock Solution section below (p.7)

**~30 to 60 min ea**

Media

Preparation of the medium is performed in two stages. (see also Example working Recipes on p.10)

I. Before autoclaving:

**~ 30 min**

1. in a 1l, autoclave proof glass bottle, mark on the side of the bottle the level of the target pre-autoclave volume (=1l, minus the volume of solutions to be added after autoclaving). For ease and accuracy, the milliQ water added to the bottle for this measurement can quantified by mass
	1. Note: if repeatedly preparing a diet of the same composition and volume, it may be easier to empirically determine the water volume displaced by the pre-autoclave solid ingredients and thus calculating the volume of water to be added. This would simplify steps 1 and 2.
2. remove ~30% of the volume for adding pre-autoclave ingredients; to be topped up later to mark on bottle made in step 1.
3. add magnetic stirrer flea to bottle
4. add sucrose and low solubility amino acids (Isoleucine, Leucine, Tyrosine)
5. add stock solutions for metal ions
6. stir well (Y may not dissolve fully at this stage)
7. add cholesterol stock solution (will not dissolve, but form a cloudy mixture)
8. add agar

II. autoclave at 120 °C for 15 min

(at the same time, sterilize any glass vials, silicone tubing for dispensing)

**~90 min**

III. after autoclaving – all steps with constant stirring

1. allow solution to cool to ~65 °C (until can just hold hands on glass bottle)

**~20 min**

1. add sterile stock solutions in the order: buffer base[[1]](#footnote-1), amino acids, vitamins, nucleosides, choline, inositol and preservatives
2. use sterile tubing is used to dispense the solution into sterile vials (in laminar flow cabinet to be extra careful about sterility, but using as close to sterile technique as possible at the bench is likely sufficient for most uses)

**~30 min**

1. either leave vials in flow cabinet to cool or transfer to bench and cover with paper towel.
2. Leave for 90 min at room temperature and then store at 4 °C until use

**90 min**

1. Tips to enhance storage (safe use ~1 month, but perhaps longer if not cracked due to drying out):
	* line a plastic tray with fresh paper towel and invert vials into tray so the opening faces down. Cover vials and seal tray with press n’seal. This both preserves the food from drying and allows any condensation on vial walls to drain out on to the paper towel during storage.
	* As much as possible, keep the tray of vials at 4C, only getting out of storage what is needed to tip your experiment.

# Equipment and reagents

## Table 1. Equipment

|  |  |  |
| --- | --- | --- |
|  | Supplier | Order number |
| silicon tubing (silastic; 4.76mm id, 7.94mm od) | VWR International  | 228-1071 |
| Autoclave |   |   |
| Schott bottles |  |  |
| Magnetic stirrer |  |  |
| Sterile syringes |  |  |
| 0.22 m syringe-mounted filter  |  |  |
| Peristaltic pumpLaminar flow hood |  |  |
| Autoclave |  |  |

##

## Table 2. Chemicals

|  |  |  |
| --- | --- | --- |
|  | supplier | cat number |
|   |   |   |
| Difco granulated agar | Appleton Woods | 214530 |
| amino acids |   |   |
| L-arginine HCl | sigma | A5131 |
| L-alanine | sigma | A7627 |
| L-asparagine | sigma | A0884 |
| L-aspartic acid | sigma | A6683 |
| L-cysteine | sigma | C1276 |
| L-glutamic acid monosodium salt monohydrate | sigma | G5889 |
| L-glutamine | sigma | G3126 |
| Glycine | sigma | G7126 |
| L-histidine | sigma | H8000 |
| L-isoleucine | sigma | I2752 |
| L-leucine | sigma | L8912 |
| L-lysine HCl | sigma | L5626 |
| L-methionine | sigma | M9625 |
| L-phenylalanine | sigma | P2126 |
| L-proline | sigma | P0380 |
| L-serine | sigma | S4500 |
| L-threonine | sigma | T8625 |
| L-tryptophan | sigma | T0254 |
| L-tyrosine | sigma | T3754 |
| L-valine | sigma | V0500 |
| sugar  |   |   |
| Sucrose | sigma | S-1888 |
| Lipid-related components |   |   |
| Cholesterol | sigma | C8667 |
| choline chloride | sigma | C1879 |
| myo-inositol | sigma | I7508 |
|  Nucleosides |   |   |
| Inosine | sigma | I4125 |
| Uridine | sigma | U3750 |
| Salts |   |   |
| acetic acid | fisher | A/0400/PB15 |
| KH2PO4 | sigma | P9791 |
| NaHCO3 | sigma | S8875 |
| CaCl2.2H2O | sigma | C7902 |
| CuSO4.5H2O | sigma | C7631 |
| FeSO4.7H2O | sigma | F7002 |
| MgSO4 (anhydrous) | sigma | M7506 |
| MnCl2.4H2O | sigma | M3634 |
| Zn SO4.7H2O | sigma | Z0251 |
|  Vitamins |   |   |
| thiamine (aneurin) | sigma | T4625 |
| Riboflavin | sigma | R4500 |
| nicotinic acid | sigma | N4126 |
| Ca pantothenate | sigma | P21210 |
| pyridoxine-HCL | sigma | P9755 |
| Biotin | sigma | B4501 |
| folic acid | sigma | F7876 |
|  |  |  |
| Propionic acid | Sigma |  |
| Nipagin M (methyl 4-hydroxybenzoate) | Clariant UK |  |

# Stock solutions

Stock solutions are generally prepared in milliQ water, except for the cholesterol stock, which is prepared in absolute ethanol. The cholesterol stock, buffer stock, amino acid solutions and stock containing nucleosides, choline and inositol are stored at 4 °C, while the FeSO4, vitamin and folic acid stocks are stored at -20 °C. Before freezing of these latter stocks, we would typically make 1 litre and make aliquots of smaller volumes so that once thawed, they could be used quickly and without re-freezing. Before storing, amino acid stocks are pH adjusted to 4.5 using HCl. All aqueous solutions were filter sterilized by passing through a 0.22 m syringe-fitted filter. Note also that cholesterol precipitates out of solution during storage at 4°C, but it easily re-dissolves with stirring and gentle heating. The amino acid ratio shown in Table 1 refers to HUNTaa (as reported in (Hunt, 1970). The amino acid proportions used to generate Yaa are determined from average values found in (Lange and Heijnen, 2001).

## 10x acetate buffer base

|  |  |
| --- | --- |
|   | g per litre |
| acetic acid | 30 |
| KH2PO4 | 30 |
| NaHCO3 | 10 |

* to ~500ml water, add acetic acid and KH2PO4, then slowly add NaHCO3 as this will froth as CO2
* make up to 1000ml with milliQ water
* pH should be ~4 at the end of all additions
* autoclave to sterilise
* store at 4C

## 66.7x stock solution

|  |  |
| --- | --- |
|   | g per 50ml |
| cholesterol | 1 |

* make up to 50ml with absolute ethanol
* store at 4C
* during storage, cholesterol will drop out of solution. Warm to 40C and shake to redissolve before adding to medium

## trace element stock solutions (6 different solutions, each at 1000x)

|  |  |  |
| --- | --- | --- |
|   | per litre |  |
| solution 1 | **1000** | **x** |
| CaCl2.6H2O | 250 | g |
|   |  |  |
| solution 2 | **1000** | **x** |
| MgSO4 | 250 | g |
|   |  |  |
| solution 3 | **1000** | **x** |
| CuSO4.5H2O | 2.5 | g |
|   |  |  |
| solution 4 | **1000** | **x** |
| FeSO4.7H2O\* | 25 | g |
|   |  |  |
| solution 5 | **1000** | **x** |
| MnCl2.4H2O | 1 | g |
|   |  |  |
| solution 6 | **1000** | **x** |
| Zn SO4.7H2O | 25 | g |
|   |  |  |

* filter sterilise each and store at room temp (sterilise due to long term storage of highly conc stocks; could also make without sterilizing and store at -20C); except for Fe solution, where:
	+ \* to avoid problems with FeSO4 rusting and dropping out of solution, aliquot and store at -20C; thaw aliquot once and store at 4C, discarding when precipitate becomes obvious.

## 125x Nucleosides and lipid-related molecules

|  |  |  |
| --- | --- | --- |
|   | per litre |   |
| choline chloride | 6.25 | g |
| myo-inositol | 0.63 | g |
| inosine | 8.13 | g |
| uridine | 7.50 | g |

* bring to 1000ml with milliQ water
* filter sterilize, aliquot and store at 4C away from light

## 47.6x vitamin solution

|  |  |  |
| --- | --- | --- |
|   | per litre |   |
| thiamine (aneurin) | 0.067 | g |
| riboflavin\* | 0.033 | g |
| nicotinic acid | 0.399 | g |
| Ca pantothenate | 0.516 | g |
| pyridoxine | 0.083 | g |
| biotin | 0.007 | g |

* bring to 1000ml with milliQ water
* filter sterilize, aliquot and store at -20C away from light
* thaw once and store any unused solution at 4C
* \* the concentration of this stock is limited by low solubility of riboflavin (Much higher concentrations possible with increased pH, but not tested effects on flies)

## 1000x Folic acid solution

|  |  |  |
| --- | --- | --- |
|   | per litre |   |
| folic acid | 0.5 | g |

* add folic acid to milliQ water, dissolve by drop-wise addition of 2M NaOH
* filter sterilize, aliquot and store at -20C away from light
* thaw once, keep any unused solution at 4C

## Amino acid stock solutions

|  |  |  |  |
| --- | --- | --- | --- |
| Amino acid | biologically available Na | HUNTaa (g/200 mlb)c | Yaa(g/200 ml) |
| Essential amino acid stock solution |
| F (L-phenylalanine) | 1 | 2.6 | 3.03 |
| H (L-histidine | 2 | 2 | 2.24 |
| K (L-lysine) | 1 | 3.8 | 5.74 |
| M (L-methionine) | 1 | 1.6 | 1.12 |
| R (L-arginine) | 2 | 1.6 | 4.7 |
| T (L-threonine) | 1 | 4 | 4.28 |
| V (L-valine) | 1 | 5.6 | 4.42 |
| W (L-tryptophan) | 1 | 1 | 1.45 |
| Non-essential amino acid stock solution |
| A (L-alanine) | 1 | 7 | 5.25 |
| C (L-cysteine) | 1 | 0.1 |  |
| D (L-aspartate) | 1 | 3.4 | 2.78 |
| G (glycine) | 1 | 6.4 | 3.58 |
| N (L-asparagine) | 2 | 3.4 | 2.78 |
| P (L-proline) | 1 | 3 | 1.86 |
| Q (L-glutamine) | 2 | 5 | 6.02 |
| S (L-serine) | 1 | 3.8 | 2.51 |
|  |  |  |  |
| Other amino acid stock solutions | ml/ld | ml/l |
| C (L-cysteine) (50mg/ml)e | 1 |  | 5.28 |
| E (L-glutamate, Na salt) (100mg/ml)f | 1 | 15.13 | 18.21 |
|  |  |  |  |
| Added as solid directly to media before autoclavingg | g/l | g/l |
| I (L-isoleucine) | 1 | 1.82 | 1.16 |
| L (L-leucine) | 1 | 1.21 | 1.64 |
| Y (L-tyrosine) | 1 | 0.42 | 0.84 |

a Moles of nitrogen available if 1 mole of amino acid completely catabolized (calculated value, not experimentally determined)

b all stock solution prepared in milliQ water, pH adjusted using HCl or NaOH as appropriate, filter sterilized and stored at 4C

c to deliver 200mM biologically available nitrogen, 60.51 ml of the essential and 60.51 ml of the non-essential amino acid solutions must be added to the medium as well as the indicated amounts of E, C, I, L, Y where they need to be added separately.

d these volumes refer to ml of stock solution to be added per liter medium.

e  For Yaa stock, C drops out during storage. A separate stock of C is made and added to food directly when adding other amino acid solutions.

# Typical working recipes:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **HUNTaa media** | 1000ml final volume |  | **Yaa media** | 1000ml final volume |
|  |  |  |  |  |  |  |  |  |  |  |
|  | mM biologically available N | **100N all** | **200N all** | **300N all** |  |  | mM biologically available N | **same total mass of amino acid as in 100N HUNTaa[[2]](#footnote-2)** | **same total mass of amino acid as in 200N HUNTaa** | **same total mass of amino acid as in 300N HUNTaa** |
|  |  |  |  |  |  |  |  |  |  |  |
| agar |   | 20 g | 20 g | 20 g |  | agar |   | 20 g | 20 g | 20 g |
|   |   |   |   |   |  |   |   |   |   |   |
| L-Ile |  Powder | 0.91 g | 1.82 g | 2.73 g |  | L-ile |  Powder | 0.58 g | 1.16 g | 1.74 g |
| L-leu |  Powder | 0.605 g | 1.21 g | 1.815 g |  | L-leu |  Powder | 0.82 g | 1.64 g | 2.46 g |
| L-tyr |  Powder | 0.21 g | 0.42 g | 0.63 g |  | L-tyr |  Powder | 0.42 g | 0.84 g | 1.26 g |
|   |   |   |   |   |  |   |   |   |   |   |
| sucrose |  50mM final | 17.12 g | 17.12 g | 17.12 g |  | sucrose |  50mM final | 17.12 g | 17.12 g | 17.12 g |
|   |   |   |   |   |  |   |   |   |   |   |
| cholesterol |  20mg/ml in EtOH | 15 ml | 15 ml | 15 ml |  | cholesterol |  20mg/ml in EtOH | 15 ml | 15 ml | 15 ml |
|   |   |   |   |   |  |   |   |   |   |   |
| CaCl2 | 1000x | 1 ml | 1 ml | 1 ml |  | CaCl2 | 1000x | 1 ml | 1 ml | 1 ml |
| MgSO4 | 1000x | 1 ml | 1 ml | 1 ml |  | MgSO4 | 1000x | 1 ml | 1 ml | 1 ml |
| CuSO4 | 1000x | 1 ml | 1 ml | 1 ml |  | CuSO4 | 1000x | 1 ml | 1 ml | 1 ml |
| FeSO4 | 1000x | 1 ml | 1 ml | 1 ml |  | FeSO4 | 1000x | 1 ml | 1 ml | 1 ml |
| MnCl2 | 1000x | 1 ml | 1 ml | 1 ml |  | MnCl2 | 1000x | 1 ml | 1 ml | 1 ml |
| ZnSO4 | 1000x | 1 ml | 1 ml | 1 ml |  | ZnSO4 | 1000x | 1 ml | 1 ml | 1 ml |
|   |   |   |   |   |  |   |   |   |   |   |
| Total vol before autoclaving |   | 780.9 ml | 712.9 ml | 644.8 ml |  | Total vol before autoclaving |   | 776.7 ml | 704.5 ml | 632.2 ml |
|   |   |   |   |   |  |   |   |   |   |   |
| buffer[[3]](#footnote-3) |  10x acetate buffer base | 100 ml | 100 ml | 100 ml |  | buffer |  10x acetate buffer base | 100 ml | 100 ml | 100 ml |
|  |  |  |  |  |  |  |  |  |  |  |
| nucl/lipid soln |  125x stock | 8 ml | 8 ml | 8 ml |  | nucl/lipid soln | 125x stock | 8 ml | 8 ml | 8 ml |
|   |   |   |   |   |  |   |   |   |   |   |
| HUNTaa solutions | essential amino acid stock solution (EAA)  | 30.26 ml | 60.51 ml | 90.77 ml |  | Yaa solutions | essential amino acid stock solution (EAA)  | 30.26 ml | 60.51 ml | 90.77 ml |
|   | non essential amino acid stock solution (NEAA) | 30.26 ml | 60.51 ml | 90.77 ml |  |   | non essential amino acid stock solution (NEAA) | 30.26 ml | 60.51 ml | 90.77 ml |
|   | Na glutamate solution (100mg/ml) | 7.57 ml | 15.13 ml | 22.70 ml |  |   | Na glutamate solution (100mg/ml) | 9.11 ml | 18.21 ml | 27.32 ml |
|   |   |   |   |   |  |   | Cys solution (50mg/ml) | 2.64 ml | 5.28 ml | 7.92 ml |
|   |   |   |   |   |  |   |   |   |   |   |
| Vitamin stock |  47.6x stock | 21 ml | 21 ml | 21 ml |  | Vitamin stock | 47.6x stock | 21 ml | 21 ml | 21 ml |
|   |   |   |   |   |  |   |   |   |   |   |
| folic acid stock |  1000x stock | 1 ml | 1 ml | 1 ml |  | folic acid stock | 1000x stock | 1 ml | 1 ml | 1 ml |
|   |   |   |   |   |  |   |   |   |   |   |
| Propionic acid |   | 6 ml | 6 ml | 6 ml |  | Propionic acid |   | 6 ml | 6 ml | 6 ml |
|   |   |   |   |   |  |   |   |   |   |   |
| Nipagin |  100 g/l stock in 95% EtOH | 15 ml | 15 ml | 15 ml |  | Nipagin |  100 g/l stock in 95% EtOH | 15 ml | 15 ml | 15 ml |

# History of updates

01/05/15

* standard procedure for timing of buffer base addition moved from before autoclaving to after. UCL appears to be the only place where adding buffer before autoclaving results in media that sets (also for MPI when they use the mediaclave), other labs need to add it after. Note that the buffer was added before autoclaving for (Piper et al., 2014) and proteome match work.

10/09/14

* Sigma have discontinued p2550 calcium pantothenate. They have recommended to replace with order number 21210. We have implemented this change and noted no change after 1 month of use

01/10/13

* FLYaa redesigned to molFLYaa

26/02/13

* under conditions of high egg laying (200N Yaa) it is probably beneficial to increase the vitamin conc by 50% from 14ml/l to 21ml/l. This appears to have no detrimental effect on egg laying (difft from previous observations since cholesterol is now higher), but enhances mid-to-late life survival of flies laying a lot of eggs. Thus, implement in all food types.

05/03/12

* increased cholesterol concentration from 100mg/L to 300mg/L since on the lower level of cholesterol at high levels of egg laying, flies were at higher risk of death early in life: this could mostly be avoided by increasing the cholesterol (also tested 200mg/L, 400mg/L and 500mg/L, which weren't optimal)

31/03/11

* slightly reduce vit increase (to 1.75x) due to higher (2.5x) levels having adverse effect on adult egg laying

22/12/10

* inc addition of vitamin (2.5x) stock - due to observed effects on these to reduce development time

17/07/07

* halved conc of all metal ion additions - no detrimental effect to egg laying or longevity (maybe even slightly improved?)

15/05/07

* reduced agar from 25g/l to 20g/l - this is the minimum for the difco agar (becomes unstable at 15g/l)

21/03/07

* reduced CaCl2 in stock due to precipitation (prob CaSO4) - reduction had no effect on egg laying

16/05/06

* iodine and cobalt additions removed from trace element solution - found to do nothing in egg laying experiments and no good evidence in the literature for their requirement (one obscure ref indicating radiolabelled iodine can be incorporated in biomass but doesn't seem to play out in practice). Vitamin B12 additions also found not have any effect and so not included.

30/03/06

* included metal ions (only Mg previously due to claim in Sang 1956 that Mg was only requirement - with extra metal ions, egg laying persisted beyond 3 weeks (without it had ceased by 3 weeks)

early development

* due to inconsistencies in Sang’s data, used choline from (Sang, 1956), adjusted inosine and uridine from (Sang, 1978) to match. Most other values from Sang cross-checked against those empirically determined from actively growing yeast (see (Lange and Heijnen, 2001))

# References

 Hunt, V. (1970). A qualitatively minimal amino acid diet for D. melanogaster. Drosophila Information Services *45*, 179.

Lange, H., and Heijnen, J. (2001). Statistical reconciliation of the elemental and molecular biomass composition of Saccharomyces cerevisiae. Biotechnology and Bioengineering *75*, 334–344.

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Sang, J.H. (1978). The Nutritional Requirements of Drosophila. (Academic Press), pp. 159–192.

Sparrow, J., and Sang, J. (1975). Physiological genetics of melanotic tumours in Drosophila melanogaster: VIII. The role of choline in the expression of the tumour gene tu bw and of its suppressor, su-tu. Genetical Research.

1. See change update notes at end of document (p. 10) for how the timing of this addition has altered [↑](#footnote-ref-1)
2. Note that for amino acid ratios other than HUNTaa, the total mass of amino acid is normalized between recipes (not the theoretical biologically available N). This means the calculated N value between diets of different ratios differs slightly, but the total mass of amino acid is identical. [↑](#footnote-ref-2)
3. Standard practice in Piper and Partridge lab until mid 2015 (includes (Piper et al., 2014)) was to add the buffer base before autoclaving. However, other labs reported this caused media not to set, a problem that was resolved by adding the buffer after autoclaving. [↑](#footnote-ref-3)