

Additions to Tissue Culture Medium

Lucy Cherbas

INTRODUCTION

Most of our cells are grown in M3+BPYE; its recipe can be found in a separate protocol. M3+BPYE is very similar to Schneider's medium, and we believe (but do not guarantee) that most of these lines will grow equally well in Schneider's medium. A few lines are grown in M3, CCM-3, or other media. Most media must be supplemented with fetal calf serum, and for many lines additional supplements are required. This protocol provides instructions on making and using these supplements. To view the requirements for any individual cell line, go to the webpage describing that line.

FETAL CALF SERUM

A few cell lines (e.g. Kc167 and S2) are very robust and will accept serum from any vendor and lot that we have tested. This is not true of most of our cell lines, particularly the CNS and imaginal disc lines. We purchase our serum from HyClone, now a subsidiary of Thermo Fisher (Hyclone US Defined Fetal Bovine (Calf) Serum, 500mL – Catalog #SH30070.03). We have made no attempt to test all available sources of serum, and there may be other suppliers whose product works equally well or better; this is simply the vendor that we use, and this serum is able to support growth of all of our serum-requiring lines. We have had good luck requesting that HyClone match a lot of serum that we have found to work well; the company keeps good analysis records of the serum lots, and is generally able to provide a good substitute for a lot that is no longer available. We are currently using lot AWC10533. Bottles of serum are stored at -20 to -30°C prior to heat-treatment. Before using a bottle of serum, thaw it, bring it to room temperature, and then heat it in a 56°C water-bath for 30-60 min. After heat-treatment, store the serum at 4°C.

Note that the temperature of serum treatment is different from that described in an earlier protocol. Many of the more robust lines (e.g. Kc, S2, S3) grow equally well in serum that is treated at 65o, but many of the more delicate



lines (e.g. disc and CNS lines) do not. We surmise that the higher temperature denatures a growth factor that is required by some of the lines.

INSULIN

All of our imaginal disc and CNS lines require addition of insulin to the medium. We use a commercial sterile insulin stock solution (Sigma I9278, supplied at 10 µg/ml); we have no evidence that this product has any particular advantages other than convenience. For the Miyake laboratory disc and CNS lines, add insulin to a final concentration of 10 µg/ml (a 1:1000 dilution of the commercial stock solution); for the Milner lab lines, add insulin to a final concentration of 5 µg/ml (a 1:2000 dilution of the commercial stock solution).

FLY EXTRACT

Some of our cell lines (imaginal disc lines made in the Milner laboratory, and ovarian lines) require the addition of a fly extract to the medium. The fly extract is not commercially available; detailed instructions on making the extract follow. This procedure is taken from a Milner lab protocol. The fly extract can be stored indefinitely at -20°. Add fly extract to medium to a final concentration of 2.5% for the Milner imaginal lines, and to a final concentration of 10% for the ovarian lines; medium containing both insulin and fly extract is stable at 4° for at least a month (D. Cottam, personal communication).

1. Flies. Collect adult flies into a capped empty vessel (we use 50 ml plastic disposable centrifuge tubes. Put them in the freezer for at least 45 minutes. They flies can be used once they are quiescent, or stored in the freezer for future use. The genotype of the flies is unimportant, as long as you avoid flies with an *ebony* phenotype; *ebony* flies have an unacceptably high level of tyrosinase. You will need approximately 300 flies (about 0.35 g) for each 2.5 ml of extract.
2. To make the extract, you will need the following items:
 - A glass homogenizer, chilled on ice
 - A refrigerated low-speed centrifuge
 - A 60° water bath.
3. Weigh the flies by transferring them to a tared tube. Transfer the flies plus 6.8 ml medium per gram of flies into a glass homogenizer, and homogenize (1 pass of the plunger is sufficient). Be careful to keep the homogenate cold, since



tyrosinase is activated during homogenization and melanization can ruin the extract.

4. Spin the homogenate at $1500 \times g$ at $4^{\circ} C$ for 15 min. Decant the supernatant into fresh tubes. Discard the pellet.
5. Incubate the supernatant at $60^{\circ} C$ for 5 min; this step will inactivate tyrosinase.
6. Spin at $1500 \times g$ at $4^{\circ} C$ for 90 min. Collect the supernatant; this is the fly extract.
7. Filter-sterilize the extract through a $0.22 \mu m$ filter. Store 2.5 ml or 10 ml aliquots at -20° .

SELECTION AGENTS USED FOR ESTABLISHMENT OF STABLY TRANSFORMED LINES

Transformed cell lines carrying a transgene are often maintained in the presence of the same selection agent that was used to establish the transformed line; this ensures that the transgene is not lost. As of this writing, our collection includes lines selected for resistance to methotrexate and for resistance to hygromycin.

Methotrexate

We purchase methotrexate from Sigma (cat. no. A6770). Dissolve the solid reagent in $0.25 M Na_2CO_3$ at a concentration of $4 \times 10^{-4} M$, sterilize by filtration through a 0.22μ filter, and store aliquots at -20° . Add the freshly thawed stock solution to medium to give a final concentration of $2 \times 10^{-7} M$ (1:2000 dilution of the stock). Medium containing methotrexate should be stored in the dark and used within 2 weeks; with time, the methotrexate loses potency as a selective agent, and it breaks down into something which is toxic even to methotrexate-resistant cells.

Warning: Methotrexate is light-sensitive. The dry reagent, stock solutions, and medium containing methotrexate must be protected from light.

Hygromycin

The following protocol based on one from the Nusse laboratory, can serve as a starting point:

We purchase hygromycin from Sigma (cat. no. H3274). Determine the actual amount of hygromycin in the bottle from the % purity listed on the label, and dissolve the contents in water to give a hygromycin concentration of 125 mg/ml. Sterilize by filtration through a 0.22μ filter, and store aliquots at -20° . A thawed



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aliquot may be stored up to a month at 4°. Add the stock solution to medium to give a final concentration of 125 µg/ml, and use the hygromycin-containing medium within a few weeks.

Note: The optimum concentration for hygromycin depends on the cell line (S. Rogers, personal communication). You may have to optimize the final concentration for the line you are working with.

Warning: Hygromycin is light-sensitive. The dry reagent, stock solutions, and medium containing hygromycin must be protected from light.

