Medium for OSS cells

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The following recipe, based on a protocol provided by Yuzo Niki, describes how we prepare OSS medium at the DGRC. Catalog numbers for components are supplied for the convenience of DGRC users; we do imply that these products are superior to those available from other venders.

As with all tissue culture media, the medium should be prepared using the highest quality water available; we use water from a Milli-Q purification system.

Recipe

For 100 ml medium, mix the following, using components described below. We store medium at 4° for up to about 2 weeks; it may be stable for longer periods than that.

- 79 ml M3 with potassium bicarbonate and potassium glutamate
- 10m heat-inactivated fetal calf serum
- 10 ml fly extract
- 1 mL 100x glutathione stock
- 100µL insulin, 10 mg/ml stock.

Although all of the components are sterile, and the medium is mixed under sterile conditions, we recommend resterilization of the completed medium by filtration through an 0.2 µ sterilizing filter because of occasional problems with residual mold spores in the fly extract. We have not found this to be necessary in media that contain a lower concentration of fly extract, but it is very helpful in this case.

Components

- 100X glutathione: 6 g L-glutathione (reduced) (Sigma-Aldrich catalog #G6013) in 100 ml H_2O, filter-sterilized, stored at 1 ml aliquots at -20°.
- insulin, 10 mg/ml: Purchase as sterile solution (human insulin , Sigma-Aldrich catalog #I9278); store at 4°.
- fly extract: Can be made according to our protocol Additions to Tissue Culture Medium, or purchased from the DGRC (click here).
- fetal calf serum (aka fetal bovine serum): Our guidelines can be found here.

Protocol: Medium for OSS cells

- M3 medium with potassium bicarbonate and potassium glutamate:
    - Dissolve M3 powder for 1 liter (Sigma Aldriche catalog #S8398) in 800 ml H_2O.
    - Add 1 g L-glutamic acid potassium salt monohydrate (Sigma Aldrich catalog #G1149) and 0.5 g $KHCO_3$..
    - Adjust pH to 6.8 with 1 M NaOH.
    - Adjust volume to 1 l.
    - Sterilize by filtration through a 0.2 μ filter.
    - Store at 4°.