

Protocol: Tissue Culture Medium
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Tissue Culture Medium

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Most of the DGRC lines are grown in M3+BPYE or M3, with variable supplements of fetal calf serum (FCS) and insulin. M3+BPYE is very similar to Schneider's medium, which is available from numerous commercial sources; some of our lines will grow if you substitute Schneider's medium for M3+BPYE, but not all.

To make M3+BPYE:

For 10 liters of medium, dissolve in 10 liters Milli-Q water:

- 393.6 g Shields and Sang powdered medium (Sigma S-8398; a bottle labeled "10 l" contains 393.6 g)
- 5 g KHCO_3

Adjust pH to 6.6. There should be little or no adjustment required.

Add and dissolve:

- 10 g yeast extract (Sigma Y-1000)
- 25 g bactopectone (Difco 211677)

Once everything is dissolved, filter-sterilize, using a 0.2 μ filter, and dispense into sterile bottles. Store at 4°C.

To prepare serum:

We buy the best available grade of fetal calf serum from HyClone; some of the lines do poorly in serum that we have tried from other vendors. 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.

