

C2C12 Cells

A. General information

C2 were originally derived from mouse thigh muscle by Yaffe and Saxel (Nature, 1977, 270, 725-7). They were later sub cloned in Helen Bleau's laboratory at Sanford. This sub clone is known as C2C12 and generally used in U.S. labs. Roy Black, in Zach Hall's lab at U.C.S.F. further sub cloned C2C12 (ca. 1983) and what you have here is one of his sub clones.

C2 cannot be passed forever; they age and eventually stop differentiating. So, it is important to amplify the cells first and make a frozen stock of the earliest passages you have available.

Although fusion of the myoblasts into multinucleated myotubes is generally considered as an obligatory correlate of skeletal muscle cell differentiation, the cells in fact start to differentiate (expressing mRNA for muscle-specific genes) when they reach confluence, even in growth medium. If you let the cells become confluent before passing them, some of them will be differentiated and may be lost. Your cultures will be progressively enriched in late or poorly differentiating cells, which is probably not what you want.

So, when you grow C2 to establish a cell stock, or any purpose that requires that the cells stay healthy over several consecutive passages, it is important not to let them grow too dense. On the other hand, they do not like to be passed often at low density. Once you have that figured out, they are not to finicky cells.

B. Handling the cells

1. Growth medium:

	Vol. For 500 ml. Bottle		final concentration
DMEM, 1 g/L glucose	400ml.	500ml.	
Fetal bovine serum (heat inactive @ 56°)	100ml.	125ml.	20%
Chick embryo extract*	2.5ml.	3.125ml.	0.5%
L-Glutamine 0.2 M**	5.0ml.	6.25ml.	2mM
Penicillin-streptomycin	5.0ml.	6.25ml.	100 U/ml-100 mcg/ml

*Chick embryo extract: from Gibco-BRL. Comes as a lyophyzed powder, each vial containing powder for 10 ml.re-hydrate with 11 ml, transfer to 15 ml conical aliquot, and store frozen at -20°C . discard the bottom pellet.

**We now use Glutamax-supplemented medium from Gibco. This additive replaces glutamine. it is claimed to be more stable and does not produce any glutamate.

2. Fusion medium:

<u>DMEM</u>	470ml.	
horse serum	25ml.	4%
L-Glutamine 0.2 M**	5.0ml.	2mM

3. Incubator conditions

8% CO_2 , 37°C , 95% humidity

4. Thawing cells

Thaw cells quickly by holding vial in 37°C water bath. Wipe vial with 70% EtOH. Use a 1ml. Pipet to transfer cells to a 15ml conical tube containing 5 ml growth medium at room t° .

Centrifuge for 5 min at 1,200 rpm and then remove the medium with a Pasteur pipette connected to a vacuum line.

Resuspend the pellet of cells in 5 ml. Growth medium and onto a T25 flask or 60 mm dish (10ml 100mm dish).

5. Splitting cells

Split the cells when they are about 70% confluent, using 0.05% trypsin-0.02%EDTA in saline (STV).

To split a T25:

- A) aspirate off the medium;
- B) wash the cells with 5ml. Serum-free medium;
- C) wash the cells with 1ml. STV;
- D) Add 1 ml. STV and wait 30 seconds to 1 min. to let the cells round up and detach (check under microscope) ; hit the side of the flask to knock off any still-attached cells (again check under the microscope);
- E) Transfer to a conical tube prefilled with 4 ml. Growth medium. The cells can now be diluted further for replating, or spun down for freezing (see further).

For C2 culture and immunostaining of the myotubes, the best way is to grow the cells on plastic chamber slides (8-well or 4-well) manufactured by Nunc. The following is the product ordering information from Fisher Scientific:

Catlog #12-565-22, 8-Well Lab-Tek Permanox Plastic Chamber Slide
Catalog#12-565-21, 4-Well Lab-Tek Permanox Plastic Chamber Slide

Everyone in the old lab of Dr. Zach Hall used these chamber slides to grow C2 and did various staining on them. Myotubes grow very well on the pre-treated plastic surface, and coating with adhesion molecules is not necessary. The chamber slides give best optical resolution and convenience. They will save you a lot of time. The drawback is the relatively high cost.

Another convenient way is to grow C2 cells on 35 mm or 60 mm dishes. Transfection may be easier to handle and AChR clusters can be viewed directly on dishes. Myotubes may be healthier due to large volume of medium. Drawbacks include 1) coating with adhesion molecules is necessary; 2) poor optical resolution due to the low Numerical Aperture of the lenses; 3) waste of antibodies due to the large volume.