

ELECTRICAL STIMULATION OF TRANSFECTED, EMBRYONIC RAT PRIMARY SKELETAL MUSCLE CELL CULTURES

Solutions and Materials:

- Teflon coated platinum wire – Medwire Part* # AG 10T, 100 feet
- Syringe needle 21G
- Bunsen burner (any flame source to heat the needle)
- Hitachi Denshi, Ltd.* Oscilloscope, Model V-212 20 MHz
- Grass Medical Instruments Photoelectric Stimulus Isolation Unit, Model PS1U6
- Grass Medical Instruments Solid-State Square Wave Stimulator (Dual Channel Square Pulse Stimulator), Model S88
- 10 K Ohm Resistor

Procedure:

1. The day after replacing the growth medium with Fusion medium check for spontaneous contraction in the Ttx-minus cultures. When spontaneous contraction is observed begin electrical stimulation.
2. Cut the necessary number of Teflon coated platinum wires to about 4cm lengths and strip the ends to remove the Teflon coating (~0.5 mm seems sufficient). These may be reused until they become too “dirty”. The ends of the wire exposed to the medium will become black. The black portion should be cut off and the Teflon striped back (if necessary).
3. Label the lids of the appropriate number of sterile, empty 60 mm culture plates and burn two holes in the lids with a heated 21G needle. Replace these lids with the appropriate lids covering the primary cultures (while in the cell culture hood).
4. Sink the wires into the plates and hook them up to the stimulator. Set the stimulator for 8 mA pulses at 50 pulses/second for a duration of one second every 100 seconds (see the “Notes Regarding Stimulator Settings” section). The trace should be solid and not a “blip”. If it is a “blip” then the circuit is incomplete and the wires require checking to ensure contact with the medium.

8mA is calculated from $V=IR$

$$I=V/R \quad I=80 \text{ V (because of the 10:1 probe)} / 10,000 \text{ Ohm} = 8 \text{ mA}$$

5. Stimulate the cells for three days prior to harvesting for Luciferase and assays. The cells should be contracting spontaneously.

NOTES REGARDING THE SETTINGS TO BE USED FOR ELECTRICAL STIMULATION OF THE PRIMARY CULTURES OF RAT SKELETAL MUSCLE CELLS:

1. The order the equipment is to be powered up in is: **Amplifier then Stimulator**
2. The limit of plates per system (amplifier :stimulator units) is 16-18 plates to reach 8mA.
3. The system should be set for 8 mA, 50 pulses per second for a duration of one second every 100 seconds.
4. The 8 mA is of alternating polarity. The **Photoelectric Stimulator Isolation Unit**, consistent current output, should have one switch set on “**Normal**” and one on “**Reverse**” (any order just so they are opposite).
5. It is set up with a 10K Ohm resistor on the photoelectric stimulus isolation unit, constant current output.
6. The **Oscilloscope** should be set on “**5 volts/ division**”.

To Set:

Train Duration: Set train duration knob to “10”
Set multiplier switch to “X100”

Train Rate: Set train rate knob to “1”
Set Multiplier to “X0.01”
Note: Move the multiplier to “X0.1” to view the amplitude on the oscilloscope (just to decrease the time necessary for set-up).

Pulse Rate: Set S1 PPS knob to “5”
Set multiplier to “X10”

Set S2 PPS knob to “5.5”
Set multiplier to “X10”

Set **S1 function** switch to “S1 train”

Set **S2 function** switch to “S1 and S2”

S1 delay – Set to “zero”

S2 delay – Set knob to “1.5”

Set multiplier to “X 10”

S1 duration – Set knob to “5”

Set multiplier to “X 0.1”

S2 duration – Set knob to “5”

Set multiplier to “X 0.1”

S1 and S2 VOLT KNOBS – Set according to the oscilloscope. Set to 8 mA.

S1 and S2 stimulus switches set to “ON”

Train mode switch set on “REPEAT”