

ELECTROPHORESIS

Method (1)

Used for precise work where the voltage across each slide is checked.

1. Warm up voltmeter
2. Set at + DC volts, 150V
3. Fill tanks with 0.05M Barbitol Buffer (pH 8.2). Mix buffer and level off.
4. Turn rheostat to "0" and put lid on switch to block inner lock.
5. Set on current regulation
6. For 1 slide, set ma to low, for 2 or more set on high (always have voltage low)
7. Check each slide individually with electrodes and regulate rheostat to give 40 volts/slide.
8. Run for 70-90 minutes.

9. Make note of setting necessary to obtain 40 volts/slide. Turn rheostat back to zero, turn power switch off and then replace lid. After this has been done, power may be turned on, rheostat returned to appropriate setting

Method (2)

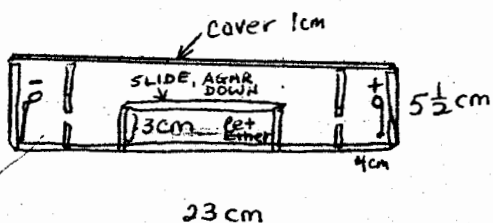
1. Eliminates use of voltmeter and checking slides
2. Run each slide at 22 ma for 4 slides 88 ma (8.8 with amps set on high) Run for 70-90 minutes.

STAINING:

After either method, stain with 1% Amido Swartz (See Ouchterlony procedure), immerse in water and wrap in bibulous paper. Dry under hair dryer, then destain in 5% Acetic Acid.

Carbonara modification of Wiene High Voltage Electrophoresis in Agar
(good protein resolution)

Materials: 1) Chamber of 23 cm x 15 cm x 5 1/2 cm



2) .9% Noble Agar in 2X Veronal buffer (.1M) pH 8.4

NaVeronal	17.0 gm
HCl 1N	23.0 ml
H ₂ O	to 1 liter


3) 1X Veronal buffer for electrode compartment

dilute 2X 1:1 with dist. H₂O

4) Petroleum Ether 40°-70° B.P.
(for cooling in chamber) Be careful = flammable

5) Agar slides

.9% noble agar in 2X strength veronal buffer
Make slides 1 mm thick and surface must be exactly flat

- In petri dish, agar underlay to make a horizontal agar surface
- Put slides down
- Pour more agar over slides to a thickness of 1 mm agar
- Carefully cut slide out of agar
-  make slits in the agar with razor blade
- Introduce serum samples with a fine tipped pipet avoiding bubbles. Sample = 2-5λ
- Invert slide in electrophoresis apparatus

Conditions of run:

150 volts for 25 min.
Approx. 30mA per slide (1" x 3")