How to use the Edvotek® Cholesterol Diagnostics Kit (#118) with The MiniOne Electrophoresis System

March 21, 2016

This guide is provided to assist teachers in adapting the lab protocol for the Edvotek Cholesterol Diagnostics Kit (#118) for use with the MiniOne Electrophoresis System. The Cholesterol Diagnostics lab introduces students to the genetics of familial hypercholesterolemia (FH) through the gel electrophoresis analysis of simulated DNA samples from five affected individuals in a family. The MiniOne produces excellent results with this lab, demonstrating separation of 4282 and 3000 bp bands within 20 minutes. Traditional electrophoresis systems require a longer run time (at least 30 minutes) or a higher voltage to produce comparable separation. The higher sensitivity of the GelGreen fluorescent stain over the FlashBlue stain supplied with the kit means that five-fold lower concentration of DNA can be used and your kit can go farther. By eliminating high voltage, UV light, and toxic ethidium bromide, safety is guaranteed and students can perform all steps of the experiment at their benches. This setup simplifies classroom management and keeps students engaged through the whole lab.

Required Materials

Reagents Supplied With Edvotek Kit:

- Concentrated 50x tris acetate EDTA (TAE) electrophoresis buffer
- QuickStrip DNA samples
- Practice gel loading solution

Other Supplies:

- 0.8% GreenGel-in-a-Cup with TAE buffer (cat. #M3140TAE)
- 0.6 mL microcentrifuge tubes (cat. #M3107 or #M3108), 8 per station
- Disposable tips for 2-20 µL micropipette (cat. #M3111)
- Distilled (DI) water

MiniOne Electrophoresis System (cat. #M1010):

- 42V power supply
- Running tank
- Carriage base
- Photo hood
- Conical flask

MiniOne Casting System (included in cat. #M1010):

- Casting stand
- Gel trays
- Gel combs

Other Equipment:

- Microwave oven
- 2-20 µL micropipettes, one per group (included in cat. #M1010, or separately cat. #M2008)
- Digital camera or cell phone with camera
- Benchtop mini-centrifuge (MiniOne Centrifuge, cat. # M2031)

Before the Lab

Prepare the running buffer

The kit is supplied with concentrated 50x tris acetate EDTA (TAE) buffer solution. Each MiniOne gel run requires 135 mL of buffer.

1) Dilute 1 volume of the concentrated 50x buffer with 49 volumes DI water.

2) Following the above calculation, add 30 mL of the concentrated buffer to 1470 mL of DI water for 1500 of 1x TAE running buffer.

3) Optional: using the conical flasks supplied with the MiniOne units, aliquot 135 mL of 1x TAE running buffer for each student group.

# Groups	Volume 50x TAE (mL)	Volume DI H_2O (mL)	Final Volume (mL)
5	17	833	850
6	19	931	950
7	22	1078	1100
8	25	1225	1250
9	27	1323	1350
10	30	1470	1500

Dilute and aliquot the DNA samples

Each tube in the Edvotek QuickStrip contains 38 µL of DNA sample. The fluorescent DNA labeling provided by MiniOne GreenGel-in-a-Cup is much more sensitive than the FlashBlue post-stain supplied with the kit. We therefore recommend diluting the samples from the QuickStrip 1:5 and using 10 µL of the diluted sample in each well. This means that each kit can be used for several classes. The following instructions are for ten student groups.

1) Label 8 microcentrifuge tubes for the samples.

2) Flick the QuickStrip with your finger to make sure the samples are well mixed.

3) Either tap the QuickStrip on the bench to make sure all of the sample is collected at the bottom of the tubes or spin down in a centrifuge using a rotor designed for strips of PCR tubes.

# Groups	Volume DNA sample (µL)	Volume practice loading buffer (µL)	Final volume (µL)
5	18	72	90
6	21	84	105
7	24	96	120
8	27	108	135
9	30	120	150
10	33	132	165

4) Using a micropipette with a disposable tip, pierce the foil covering of the QuickStrip tube, withdraw the required amount of sample and dispense into a microcentrifuge tube. Repeat for all 8 samples.

5) To each microcentrifuge tube add the required amount of practice gel loading solution. Flick or vortex to mix the dye and DNA sample, then tap on the bench or centrifuge to collect all of the diluted sample at the bottom of the tube.

6) Label a set of 8 microcentrifuge tubes for each of the student groups.

7) Aliquot 15 µL of each diluted sample into the labeled microcentrifuge tubes.

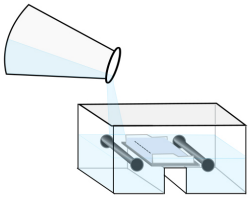
Set up stations for each student group

- 1 MiniOne Casting System
- 1 MiniOne Electrophoresis System with photo hood
- 1 of the 0.8% GreenGel-in-a-Cup with TAE buffer
- 8 DNA sample aliquots
- 1 micropipette (2-20 µL) and pipette tips

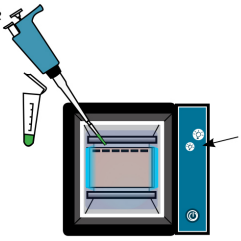
MiniOne Gel Loading and Running Instructions:

How to Load a Gel

1. Put gel in the tank matching the wells on the platform. Measure 135 mL of 1X TAE running buffer and pour into **one** side of the tank to push out the air, creating a nice even background without air bubbles or air trapped for imaging later.

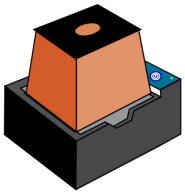


2. Turn the low intensity blue light on by pressing the button on the carriage to help visualize the wells when loading. Load 10 µL per well. Remember to change pipette tips for each sample. **Take care mot to damage of puncture the wells**

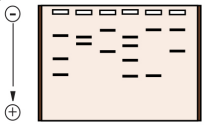


Visualize and Capture Image

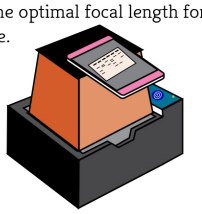
3. Once the gel is loaded, do not move it. Make sure the power supply is plugged in and place the photo hood on the carriage. Turn on the unit by pressing the button. The green LED next to the button will turn on.



4. Allow the gel to run approximately **20 mins** or until DNA separation is sufficient. After your run is complete, turn off the power by pressing the button. Use the low intensity for viewing during the 20 mins. Light will weaken the fluorescent DNA signal.



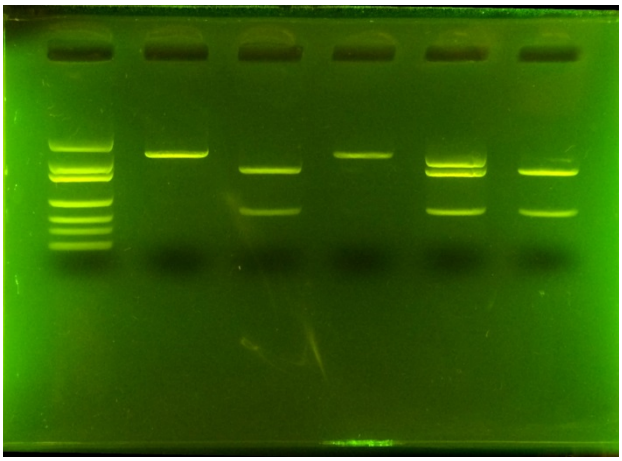
5. **Document your results.** Wipe off condensation from the inside of the hood with a soft cloth. Place your cell phone or camera directly on the photo hood to take a picture of the DNA. The photo hood is already at the optimal focal length for a smart device.



Example Results:

Edvotek kit DNA samples diluted
Lane 1: Molecular weight marker
Lane 2: Normal control DNA sample
Lane 3: FH homozygote control
Lane 4: DNA sample 1
Lane 5: DNA sample 2
Lane 6: DNA sample 3

After 20 minutes DNA band separation is visible in all samples.



Purchasing Information:

0.8% GreenGel-in-a-Cup with TAE buffer: Cat. #M3140TAE
MiniOne Centrifuge: Cat. # M2031
MiniOne Pipette 2-20 µL: Cat. #M2008
MiniOne Electrophoresis Units Classroom Package: Cat. #M1010
0.6 mL Microcentrifuge Tubes, Rainbow: Cat. #M3108
Universal Fit Pipette Tips: Cat. #M3111