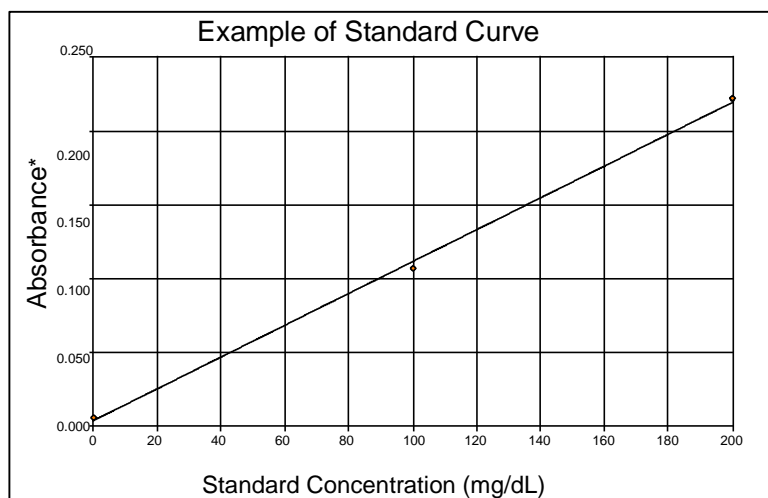


## RUO Microplate/Manual Applications

### Free Cholesterol E Microtiter Procedure

For Research Use Only. Not for use in diagnostic procedures.  
Cat. No. 993-02501(kit) **Free Cholesterol E**

1. Prepare working Color Reagent Solution and standards according to the package insert instructions.
2. Pipette 5  $\mu\text{L}$  of each of the following into the assigned microplate well according to plate layout: saline (to be used as blank), each level of standard, and sample.
3. Add 300 $\mu\text{L}$  of Color Reagent Solution.
4. Mix well and incubate at 37°C for 5 minutes.
5. Measure the absorbance of each well at 600nm.  
Use 700nm as the reference or secondary wavelength\*.  
Absorbance readings should be completed within 1 hour.
6. Plot the absorbance vs. concentration for the standard (calibration) curve.  
A Linear Calculation model should be used.  
(Refer to package insert for further details on the manual calculation of results).



7. Refer to the package insert for calculation of ester ratio.

*\*Note: The secondary absorbance reading at 700nm is only for bichromatic measurement to correct for any serum interferences. Absorbance readings taken at 700nm should be subtracted from the absorbance readings taken at 600nm.*