

Bauer Core Standard Protocol		
Title: Using the Spectramax Gemini XS Fluorescent Plate Reader		
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1. Purpose

This protocol provides instructions for using the Spectramax Gemini XS Fluorescent Plate Reader to capture fluorescent or luminescent data from microtiter plates at wavelengths between 250 and 850 nm. The protocol is designed as a reference and is not a substitute for training. Users must complete a training session before using any of the Bauer Core's instrumentation.

2. Materials

- 2.1. Opaque microtiter plate (eg Costar plate sold by VWR cat # 29444-018)
- 2.2. Fluorescent or Luminescent samples (100ul volume)

3. Instrumentation

Molecular Dynamics Spectramax Gemini XS

4. Reagent preparation

none

5. Procedure

- 5.1. Open the SoftMax Pro software using the Spf shortcut on the desktop.
- 5.2. Click on the Instrument Icon.
 - 5.2.1. Choose COM 3 and Gemini XS.
- 5.3. Click on Plate 1 – Setup.
 - 5.3.1. Assay type.
 - 5.3.1.1. Endpoint: most common assay, 1 scan/well, up to 4 wavelengths.
Use autocutoff to ensure excitation wavelength isn't detected.
 - 5.3.1.2. Kinetic: will scan several times over selected run time.
Choose run time and interval.
 - 5.3.1.3. Spectrum: will scan over a range of wavelengths.
 - 5.3.1.4. Well Scan: will take several scans within one well.
 - 5.3.2. Read Type: choose Fluorescent or Luminescent assay.
 - 5.3.3. Sensitivity: use slide bar to choose faster scan or more precise scan.
use PMT = automatic.

- 5.3.4. Automix: will shake the plate for 5s (not very powerful).
- 5.3.5. AutoCalibrate: on.
- 5.3.6. Assay Plate Type: choose from the list of well types.
note that the plate must be opaque (see section 2.1).
- 5.3.7. Wells to read: select wells in use.
- 5.3.8. Autoread: off.
- 5.4. Click on Template.
 - 5.4.1. Choose which wells are blanks, standards and unknowns.
Blanks will not be automatically subtracted – raw data will be presented.
To subtract blanks, copy and paste data into excel and do calculations.
- 5.5. Click on Reduction.
 - 5.5.1. Use plate blank (there is no pre-read option for the fluorimeter).
 - 5.5.2. Input any formulas you would like to use.
- 5.6. Click on Display to determine what data is shown (raw only or reduced).
- 5.7. Click on Temperature to set the assay temperature from 4°C above ambient to 45°C.
- 5.8. Place your plate in the reader.
 - 5.8.1. Click the “drawer” button on the instrument to open and close the drawer.
 - 5.8.2. Well A1 should be in the back left corner.
- 5.9. Click Read.
- 5.10. Copy and paste the results into excel for analysis.
- 5.11. To enter another plate click Experiment – New Plate .
- 5.12. To save the settings, click Save As.
this will save a .pda file (readable only in softmax).
- 5.13. Remove your plate, close the drawer, and log off from the computer when finished.