

Measuring Fluorescence from Cy[®]3 Dye using the GloMax[®] Discover System

Promega Corporation



Materials Required

- GloMax[®] Discover System (Cat.# GM3000)
- Nuclease-Free Water (Cat.# P1195)
- single-stranded RNA (ssRNA) labeled with Cy[®]5 dye, rehydrated to 100 μ M in water (Integrated DNA Technologies)
- ssRNA labeled with Cy[®]3 dye, rehydrated to 100 μ M in water (Integrated DNA Technologies)
- black, solid-bottom 96-well assay plates (Greiner Cat.# 655076)

Caution: We recommend the use of gloves, lab coats and eye protection when working with these or any chemical reagents.

Protocol: *GloMax[®] Discover System*

Technical Manual #TM397 is available at:

www.promega.com/protocols/

Cyanine (Cy) dyes are commonly used for applications involving DNA, RNA or protein labeling. Detection of these dyes can be accomplished using image-based methodologies, fluorescence scanners or microplate instrumentation. Cy[®]3 and Cy[®]5 dyes are commonly used together for two-color detection in a sample due to their distinct spectral properties (Cy[®]3 = orange, Ex: 554nm, Em: 568nm; Cy[®]5 = far-red, Ex: 649nm, Em: 666nm)

Cy[®]3 quantification is made easy on the GloMax[®] Discover System. The extended dynamic range and minimal well-to-well cross talk of the GloMax[®] Discover System allows you to easily measure signals of varying intensities on the same plate. This Application Note describes the protocol for measuring fluorescence from Cy[®]3 dye using the GloMax[®] Discover System. Here we highlight the ability of GloMax[®] Discover to detect fluorescence signal from a titration of Cy[®]3 dye alone (Figure 1) or from a mixture of Cy[®]3 and Cy[®]5 dyes (Figure 2).

Cy[®]3 Dye Protocol

Protocol 1: Cy[®]3 Dye Titration

1. Prepare a 1 μ M working stock of Cy[®]3 dye by combining 10 μ l of 100 μ M stock with 990 μ l of Nuclease-Free Water. Invert to mix.
2. Prepare a twofold dilution series of the 1 μ M working Cy[®]3 solution. Include a water-only control for background.
3. Transfer 100 μ l of each dye dilution to quadruplicate wells of a black 96-well plate.
4. Measure Cy[®]3 fluorescence using the [Ex: 520nm, Em: 580–640nm] filter set on the GloMax[®] Discover System using the Instrument Protocol.

Instrument Protocol

1. Create a New Protocol on the GloMax[®] Discover System.
2. Add a fluorescence measurement step into the protocol.
3. Select Excitation 520nm from the Excitation drop-down menu.
4. Select Emission 580–640nm from the Emission drop-down menu.
5. Save protocol changes.
6. Add the plate to the instrument, and Start the protocol.

The GloMax[®] Discover System detected Cy[®]3 fluorescence over 3.5 orders of magnitude of Cy[®]3 concentration. The approximate limit of detection, defined as three standard deviations above background, for Cy[®]3 dye was 240pM (dashed line).

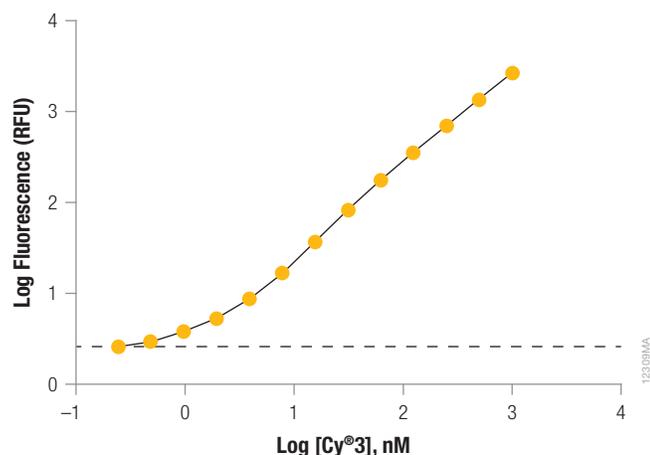


Figure 1. Concentration of Cy[®]3 dye detected using GloMax[®] Discover System. A twofold serial titration of Cy[®]3 dye was prepared in nuclease-free water, and 100 μ l of sample was transferred in replicates of four to a black 96-well microplate. The green filter set was used for detecting Cy[®]3 dye fluorescence.

Protocol 2: Cy[®]3/Cy[®]5 Dye Mixture Study

1. Prepare a 250nM working stock of Cy[®]3 dye by combining 5 μ l of 100 μ M stock with 1.995ml of Nuclease-Free Water. Invert to mix.
2. Prepare a 250nM working stock of Cy[®]5 dye by combining 5 μ l of 100 μ M stock with 1.995ml of Nuclease-Free Water. Invert to mix.
3. Create eleven Cy[®]3/Cy[®]5 dye mixtures containing varying percentages of each Cy[®] dye in a total volume of 350 μ l (see Figure 2). Include Cy[®]3 dye-only and Cy[®]5 dye-only controls.
4. Transfer 100 μ l of each dye mixture to triplicate wells of a black 96-well plate.
5. Measure Cy[®]3 fluorescence using the [Ex: 520nm, Em: 580–640nm] filter set and Cy[®]5 fluorescence using the [Ex: 627nm, Em: 660–720nm] filter set of the GloMax[®] Discover System.

Results show that the percentage of fluorescence emitted from each dye (Y axis) is in close agreement with the actual percentage (X axis) of Cy[®]3 and Cy[®]5 dyes added to the well.

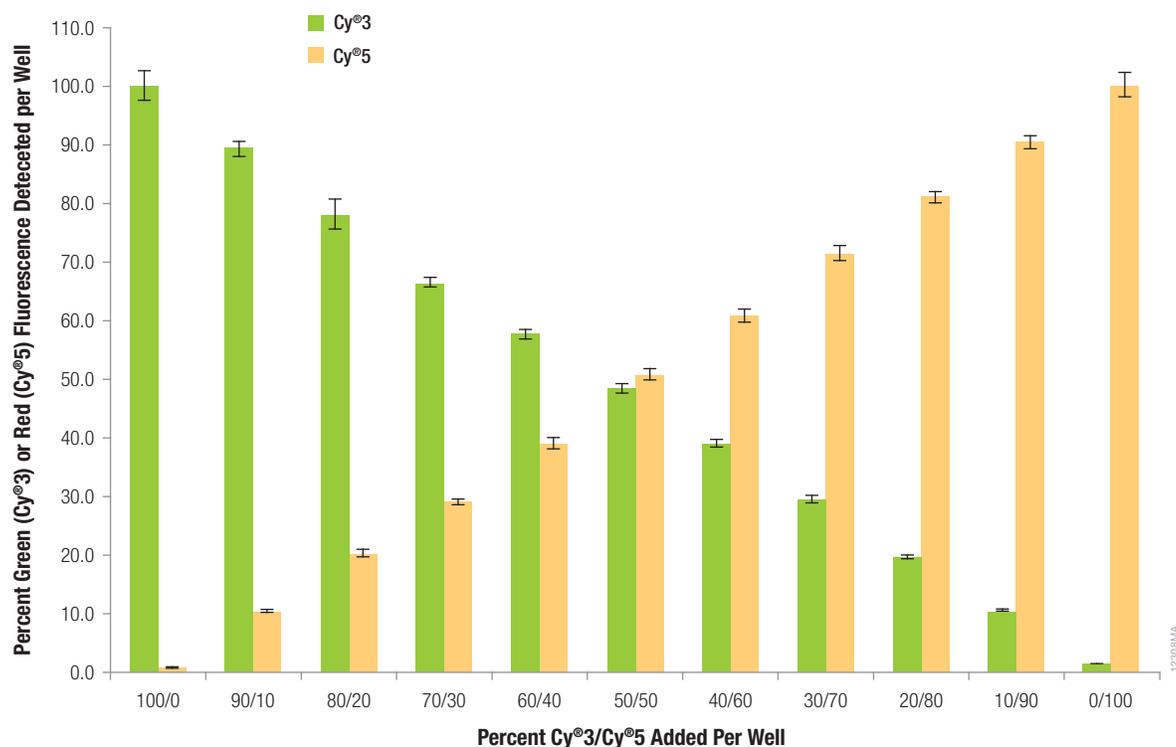


Figure 2. Cy³ dye can be detected in a mixture containing Cy⁵ dye. 250nM solutions of Cy³ and Cy⁵ dyes were prepared and combined to create various ratios of Cy³ dye and Cy⁵ dye. Triplicate 100µl aliquots of each solution were added to wells of a black 96-well plate. Fluorescence was detected from all wells using the green and red filter sets.

Conclusion

This Application Note demonstrates that the GloMax[®] Discover can measure fluorescence from the Cy³ dye.

The GloMax[®] Discover System

The GloMax[®] Discover System offers superior sensitivity and dynamic range and limited well-to-well cross talk. The instrument was developed and optimized with Promega cell and gene reporter assays and may be integrated into low- and medium-throughput automation workflows. The GloMax[®] Discover System allows flexible use of filters to measure fluorescence intensity, filtered luminescence, BRET, FRET and UV-visible absorbance for a wide variety of laboratory applications. The instrument is operated by an integrated Tablet PC, which provides quick and easy navigation through the control options. Exporting your results is made seamless with a variety of options, including exporting data to your local network.

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