

RediPlate™ Microplate Intensity Standards

Quick Facts

Storage upon receipt:

- Room temperature
- Protect from light

Abs/Em: See Table 1 and Figure 2

Introduction

RediPlate™ microplate intensity standards provide stable and consistent reference materials for standardization of fluorescence measurements in 96-well microplates. Each standard consists of a conventional 96-well microplate containing a fluorescent dye suspended in a proprietary protective matrix. The standards generate a linear series of fluorescence intensities covering three orders of magnitude in twofold increments (Figure 1) and are suitable for use with both top- and bottom-reading fluorescence-based microplate readers. Standards are available with different fluorescent colors matching the spectral characteristics of dyes and probes commonly used in fluorescence-based microplate assays (Table 1).

A RediPlate 96 microplate intensity standards set for green, orange, red and far-red fluorescence (R36910) containing one 96-well microplate each of the green (R36901), orange (R36902), red (R36903) and far-red (R36904) fluorescent microplate standards is also available.

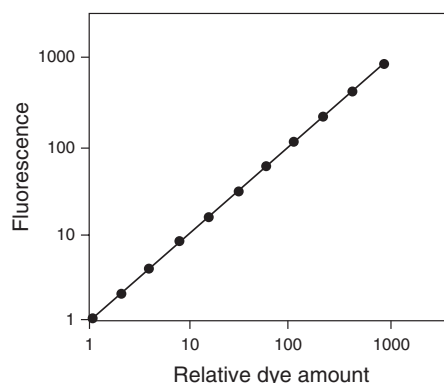
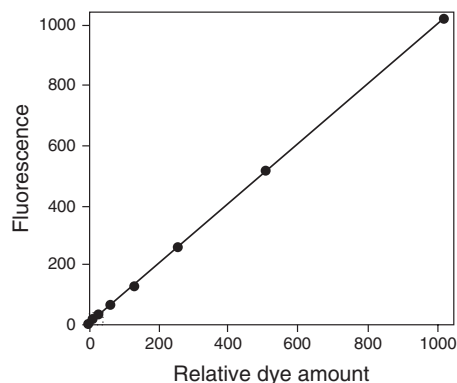


Figure 1. Typical data from the green-fluorescent RediPlate microplate intensity standard obtained on a fluorescence-based microplate reader using excitation at $485 \text{ nm} \pm 10 \text{ nm}$ and emission detection at $530 \pm 12.5 \text{ nm}$. Each data point represents the average of eight readings representing the eight replicate wells in each column of the standard microplate. Error bars (± 1 standard deviation for eight replicate readings at each relative dye amount) are smaller than the plotted symbols and have therefore been omitted. Panel A shows the full analytical range plotted on linear axes. Panel B shows the same data, with background values subtracted, plotted on logarithmic axes to emphasize the linear fluorescence intensity range of the standards spanning three orders of magnitude.

Table 1. RediPlate microplate intensity standards.

Catalog Number	Fluorescence Color	Abs/Em *	Spectrally Compatible Dyes and Probes
R36901	Green	490/510	Alexa Fluor® 488 dye, fluorescein (FITC), Oregon Green® 488 dye, BODIPY® FL dye, EGFP, PicoGreen® dye †, RiboGreen® dye †, OliGreen® dye †
R36902	Orange	565/580	Alexa Fluor 555 dye, Alexa Fluor 546 dye, Alexa Fluor 568 dye, BODIPY TMR dye, Cy™3 dye, tetramethylrhodamine (TRITC)
R36903	Red	590/605	Alexa Fluor 594 dye, Texas Red® dye, BODIPY TR dye
R36904	Far red	660/675	Alexa Fluor 647 dye, Cy5 dye

* Approximate absorption (Abs) and fluorescence emission (Em) maxima, in nm; full absorption and fluorescence emission spectra are shown in Figure 2. † When bound to nucleic acid.

Materials

Contents

RediPlate microplate intensity standards are ready to use as supplied. DO NOT add liquids or media of any sort to the standard microplate wells, as this will cause permanent damage to the fluorescent coating, thereby invalidating the standard. Do not scratch or otherwise physically disrupt the coatings on the inside surfaces of the microplate wells.

The layout of the standard 96-well microplate is described in Table 2. The eight rows (A–H) of the microplate contain replicate fluorescence intensity progressions covering three orders of magnitude in twofold steps. The dye amounts in each of the eight wells (A–H) in a given column are nominally equal. However, minor variations occur as a normal result of the manufacturing process.

The standards are extremely durable under normal use conditions. Typically, no more than a 4% decrease in fluorescence intensity is observed in a single well after 100,000 consecutive readings using a conventional fluorescence-based microplate reader.

Storage

When not in use, RediPlate microplate intensity standards should be stored at room temperature, protected from light.

Experimental Protocol and Results

1. Set up the microplate reader by selecting suitable excitation and emission filters and setting the photometric gain/sensitivity. The absorption and fluorescence emission spectra shown in Figure 2 can be used as a guide for filter selection. The photometric gain/sensitivity setting should be such that signals from the highest-reading wells (A1–H1) do not saturate the detector. If the standard is to be used as a reference for an assay readout, the excitation and emission filters and the photometric gain/sensitivity setting should be identical to those used for the assay [Note A].

Table 2. Layout of RediPlate 96 microplate intensity standards.

Column	Relative Dye Amount *
1	1024
2	512
3	256
4	128
5	64
6	32
7	16
8	8
9	4
10	2
11	1
12	0 (background)

* Fluorescence intensity is directly proportional to the amount of dye (see Figure 1).

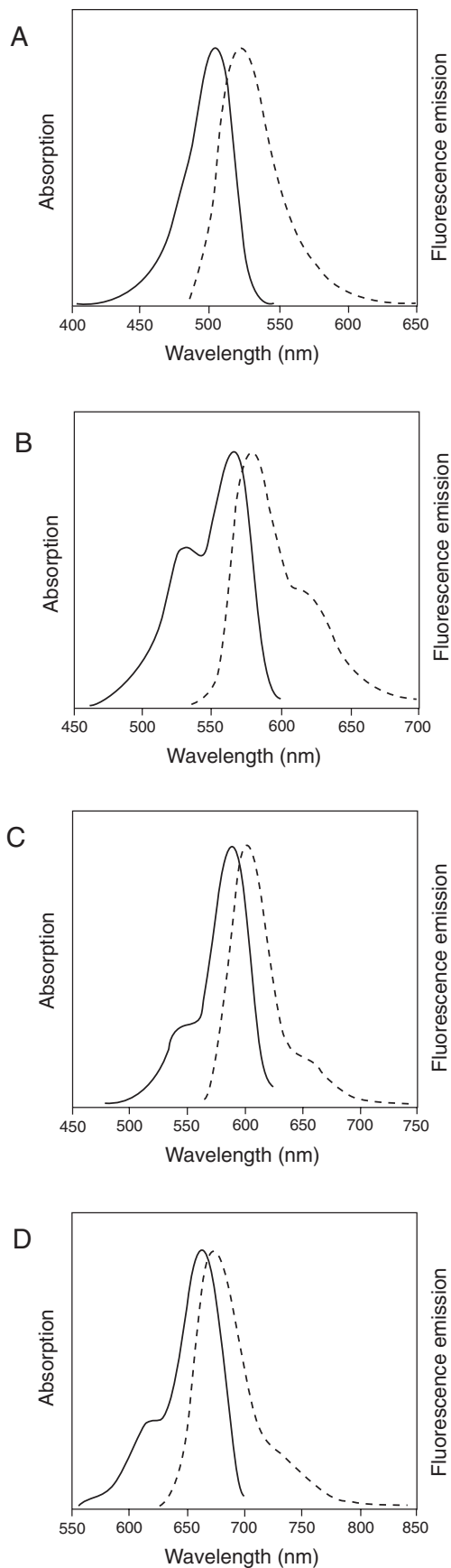


Figure 2. Absorption and fluorescence emission spectra for the A) green, B) orange, C) red and D) far-red RediPlate microplate intensity standards.

2. Most microplate reader control programs have input settings that define the physical dimensions of the microplate. Make sure this input value is consistent with the dimensions of the standard microplate.

3. If the temperature of the microplate reader sample chamber is significantly different from room temperature, the standard microplate will need to be thermally equilibrated in the chamber for at least 30 minutes prior to reading. Alternatively, the standard microplate can be thermally equilibrated at room temperature and read very quickly (within 60 seconds or less) so that the temperature of the plate does not change significantly before completion of the reading [Note B].

4. Read the fluorescence intensities of all 96 wells on the standard microplate.

5. Analyze the data. A typical linear regression analysis is shown in Figure 1. Background signal values (wells A12–H12) may be subtracted from fluorescence signal values, if desired.

6. The data should yield a linear regression fit parameter $R^2 \geq 0.99$. Linear regression slopes from repeat readings of the standard microplate should have a coefficient of variation of $\leq 2\%$ (assuming that the excitation and emission wavelength and photometric gain/sensitivity settings are unchanged).

Notes

[A] The fluorescence intensities of the RediPlate microplate intensity standards correspond very approximately to 100–200 μL volumes of dye solutions with concentrations of 1–1000 nM. Actual correspondence depends on the excitation and emission wavelength filters used for the measurements and the molar extinction coefficient and fluorescence quantum yield of the dye in solution.

[B] The fluorescence intensities of the standard microplate dye samples decrease slightly with increasing temperature. Therefore, to obtain reproducible standard data, it is important to maintain the run-to-run consistency of the reading temperature.

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
R36901	RediPlate™ 96 microplate intensity standards, green fluorescent (505/520) *one 96-well microplate*	each
R36902	RediPlate™ 96 microplate intensity standards, orange fluorescent (565/580) *one 96-well microplate*	each
R36903	RediPlate™ 96 microplate intensity standards, red fluorescent (590/605) *one 96-well microplate*	each
R36904	RediPlate™ 96 microplate intensity standards, far red fluorescent (660/675) *one 96-well microplate*	each
R36910	RediPlate™ 96 microplate intensity standards set *includes one each of R36901, R36902, R36903 and R36904*	1 set

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