DNA quantification with Pico488

Pico488 DNA quantification solution is an ultrasensitive reagent to measure concentration of double-stranded DNA if that cannot be determined by measuring the absorbance at 260 nm. Pico488 selectively binds to double-stranded DNA, so nucleotides, single-stranded DNA, RNA, proteins, and other impurities do not impede the measurements. The structure of the dye is identical to that of PicoGreen™ dye. The dye bound to double-stranded DNA absorbs light with maximum at 503 nm and emits light with maximum at 525 nm. To detect the assay read-out, any type of fluorometers or fluorescence plate readers can be used.

The range of linearity for DNA concentration measurements with Pico488 is 1 pg/µL — 5 ng/µL. For precise reproducible fluorescence measurements, we recommend to dilute DNA and Pico488 in TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). To calculate DNA concentration we recommend first to build a calibration curve using a series of DNA standard dilutions.

Lumiprobe sells Pico488 DNA quantification solution in various packs and Pico488 DNA quantification kit. The kit includes both buffer and DNA standard stock solution in addition to the Pico488 solution. The amount of dye solution supplied with the kit is sufficient to analyze 200 experimental data points in a standard fluorometric cuvette (volume 3.5 ml), if the assay volume is equal to 2 ml. The assay can be rescaled if other types of equipment are used to detect fluorescence. Recommended assay volumes for commonly used fluorometric equipment are provided in the table below.

Protocol

1. Preparation of Pico488 working solution

Thaw the content of Pico488 dye vial, mix thoroughly, and dilute a needed amount of the Pico488 dye solution 200-fold with a buffer (we recommend to use 10 mM Tris HCl, 1 mM EDTA, pH 7.5). Mix and use within 3 hours. The volume of Pico488 working solution for each experimental data point should be equal to 50% of assay volume (check the table below to find the recommended volumes for your type of fluorometric equipment). Prepare enough Pico488 working solution for all experimental data points (for all samples and all DNA standard dilutions that you plan to analyze). Include also additional 10-25% of Pico488 working solution volume to exclude possible pipetting errors. To calculate the volume of diluted Pico448, you can use the following formula:

$V_{Pico488} = 5/8 \times V_{assay} \times (N_{samples} + N_{standards})$, where V_{assay} is the assay volume of a sample or a standard, mL, $N_{samples}$ is the number of samples that you plan to analyze, and $N_{standards}$ is the number of standards that you plan to analyze (including blank sample).

2. Preparation of sample solution

Dilute your DNA sample in the buffer to get the volume of solution equal to 50% of assay volume (you may take any amount of DNA). Add equal volume of Pico488 working solution. Mix and incubate for 5 min. Likewise, prepare dilutions of DNA standard. Please, note that the dilutions of DNA standard should be within the range of DNA concentrations in your sample. The DNA standard stock solution is only provided with Pico488 DNA quantification kit. The users of Pico488 DNA quantification solution should use their own DNA standards.

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Recommended volumes for DNA quantification with Pico488 DNA quantification solution:

Type of equipment	Assay volume	Volume of diluted Pico488	Volume of diluted DNA
Standard fluorometric cuvette (3.5 ml)	2 ml	1 ml	1 ml
Other fluorometric cuvettes	About 75% of cuvette volume	37.5% of cuvette volume	37.5% of cuvette volume
96-well plate*, per well	0.2 ml	0.1 ml	0.1 ml
24-well plate, per well	1 ml	0.5 ml	0.5 ml
Other plates	About 75% of well volume	37.5% of well volume	37.5% of well volume
NanoDrop™ 3300*	0.1 ml	0.05 ml	0.05 ml

* To maintain accuracy and precision of your measurements, we recommend to avoid pipetting volumes below 2 µL.

3. Fluorescence measurements

Measure fluorescence from standard and sample DNA solutions using appropriate absorption and emission wavelengths or filters (double-stranded DNA bound Pico488 dye absorbs light with maximum at 503 nm and emits light with maximum at 525 nm).

4. Calculation of DNA concentration

Plot fluorescence *vs* concentration and apply linear regression function in any software to obtain linear equation reflecting fluorescence (*FL*) vs concentration (*C*) dependence:
$FL = A \times C + B$.

To calculate DNA concentration in the diluted sample use the following formula:
$C_{sample} = (FL_{sample} - B)/A$, where FL_{sample} is sample solution fluorescence.

To calculate DNA concentration in your undiluted sample use the following formula:
$C_{init} = V_{assay} \times C_{sample} / V_{init}$, where V_{assay} is the assay volume, mL and V_{init} is the volume of your initial DNA sample, µL

Alternatively, you can use our dsDNA quantification and dilution calculators to have all necessary calculations done.

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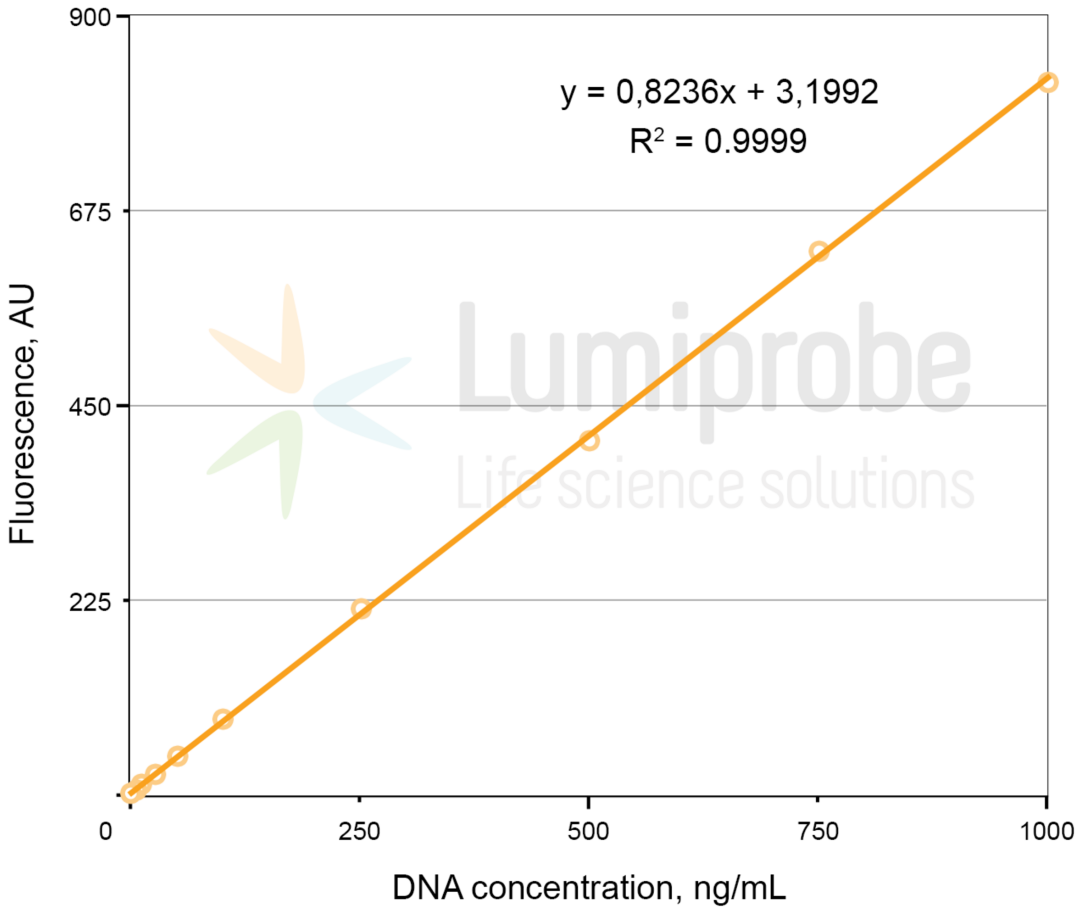
Linear regression example: fluorescence versus DNA concentration



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