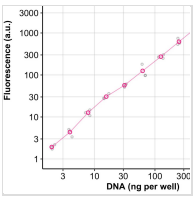

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Microplate-based DNA Quantification with EzFluoroStain DNA reagent

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This protocol offers an safer alternative to the ethidium bromide-based DNA quantification protocol, utilizing an DNA-selective dye EzFluoroStain DNA (WSE-7130, ATTO corporation, Tokyo, Japan). This protocol allows to quantify about 2-1000 ng DNA/well, using a standard fluorescent plate reader.

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DNA quantification, DNA assay, microplate protocol, molecular biology, fluorescence

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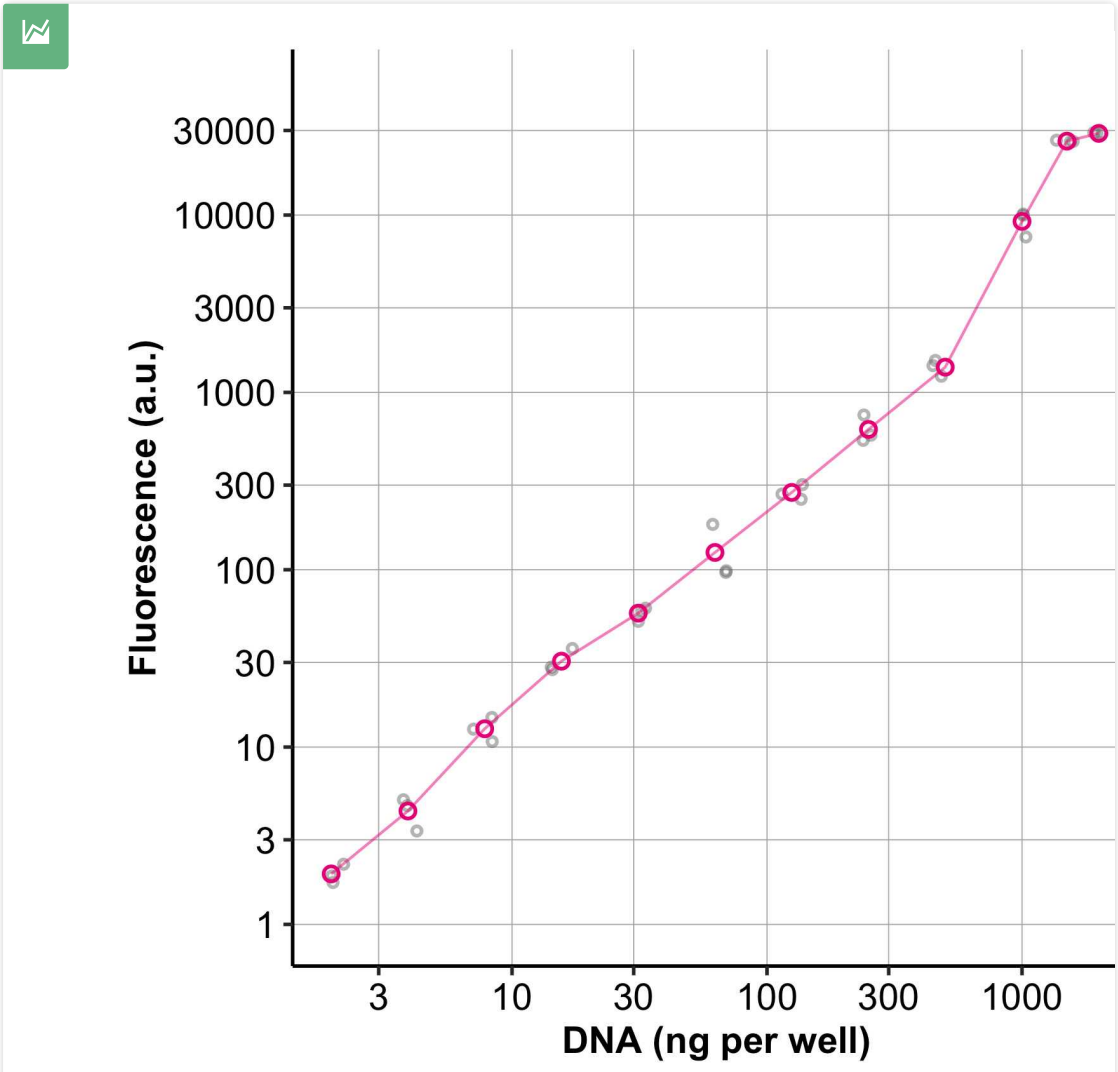
This DNA quantification protocol is based on interaction of a fluorogenic dye EzFluoroStain DNA (ATTO Corporation, Japan) with DNA. The exact structure of this commercial dye is not disclosed, the mechanism is similar to intercalating dyes ethidium bromide (Bonasera et al., 2007), SYBR Green (Leggate et al., 2006), and PicoGreen (Singer et al., 1997). Although some commercial reagents for DNA quantification, including PicoGreen, offer higher sensitivity, this dye can be a cheaper alternative sufficient for routine use. In our laboratory, we routinely use EzFluoroStain DNA for both DNA quantification and agarose gel staining.

Compared with UV-based measurement, this protocol allows determination of lower concentration of DNA, with relatively small amount of the DNA solution (1-10 µL).

This protocol allows the users to quantify about 2-1000 ng DNA per well. However, the dynamic range may vary depending on different plate readers and settings (especially for lower concentration range). The detection settings/methods may need to be optimized for this assay, using standard samples.

In the protocol, 5 µL sample / well was used as a standard condition. However, the amount of the sample can be varied depending on the estimated concentration of the samples. For example, 0.4 to 200 ng/µL DNA solution can be measured with this 5 µL/well protocol, and 2 to 1000 ng/µL DNA solution can be assayed with 1 µL/well protocol. Note that more than 1000 ng DNA per well results in saturation of the fluorescence, as shown in the example data below.



An example of DNA quantification with this assay.
Serial dilution of a plasmid of known concentration (determined by UV absorbance) was prepared in Tris-EDTA buffer (pH 8.0), and 5 µL of the solution was mixed with 95 µL of EzFluoroStain DNA working solution (1/1000 in Tris-EDTA buffer) on a all-black 96-well plate. Fluorescence (Ex. 475 nm / Em 500-550 nm)

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was measured on a GloMax Explorer (a multi-mode, filter-based plate reader), and background fluorescence was subtracted. The experiment was performed in triplicate samples and raw data and their average was shown as magenta circles and gray circles, respectively.

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Equipments

GloMax Explorer
multi-mode plate reader
Promega **GM3500**

Any microplate reader equipped with fluorescence mode (Excitation around 475 nm and emission 500-550 nm) would be fine.

Reagents

- EzFluoroStain
- DNA **ATTO Catalog #WSE-7130** Store at -20ºC, protect from light.
- 1X TE buffer (10 mM Tris-HCl pH 8.0 1 mM EDTA) **Contributed by users** Also, standard Tris-Acetate-EDTA buffer (TAE buffer) commonly used for agaraose gel electrophoresis is compatible with this assay.
- Nunc™ F96 MicroWell™ Polystyrene Plate, cell culture, black **Thermo Fisher Catalog #137101**
  Half-area plates and 384-well plates would also be OK. Alternatively clear 96-well plate may also be used.

Although the EzFluoroStain DNA dye is claimed to be safer than ethidium bromide based on the Ames test, it still binds to DNA and is a potential carcinogen. So care must be taken when handling this reagent, and the reagent waste needs to be properly disposed of.

We recommend to aliquot EzFluoroStain DNA reagent in ~10µL and minimize freeze-thaw cycles (several freeze-thaw cycles are OK). Before starting the experiment, thaw an aliquot of EzFluoroStain DNA reagent while protecting from light.

Sample preparation

1 **Prepare dilution series of standard DNA solution, depending on the concentration of your sample.**

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To make full-range standard curve (~4 ng/well to 1000 ng/well), prepare 200, 100, 50, 25, ... , 1.56, 0.78 ng/µL in Tris-EDTA buffer. For routine use, 3-4 points may be sufficient (e.g., 200, 20, 2ng/µL).

2 **Prepare EzFluoroStain DNA working solution.**

Thaw an aliquot of EzFluoroStain DNA at room temperature and dilute EzFluoroStain DNA solution by 1000-fold in Tris-EDTA buffer and mix by vortex. Prepare required amount of the solution (at least 95 µL/well is needed).

3 **Mix 5 µL of the DNA samples with 95 µL of the EzFluoroStain DNA working solution on a black 96-well microplate.**

The volume of the DNA sample can be varied depending on the estimated concentration of the sample. 1 µL/well (for higher concentration samples) to 10 µL/well (for lower concentration samples) may be used. Please adjust the volume of the working solution so that the total volume becomes 100 µL (for standard 96-well plates). Although the fluorescence can be measured soon after mixing the solution, it might be better to wait for 3-5 min to equilibrate the dye-DNA binding. The fluorescence is stable at least for 30 min at room temperature. Although all-black microplates are recommended for higher sensitivity, standard clear microplates may also be used.

Measurement & analysis

4 **Measure fluorescence.**

The excitation/emission maximum are reported to be around 495 nm and 522 nm. For filter-based fluorimeter, excitation filter around 475 nm and band-pass emission filter 500-550 nm is recommended. For monochrometer-based fluorimeter, it would be better to use the maximum wavelength for excitation/emission settings.

5 **Calculate the concentration of your samples using standard curve.**

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