

PicoGreen dye-binding assay for the quantitation of Adenovirus DNA

Description

Molecular Probes PicoGreen dye is a sensitive fluorescent DNA binding dye, selective for double stranded DNA over single stranded DNA and RNA. Adenovirus type 5 (Ad5) DNA is quantitated in the following manner: the sample is pretreated with a combination of DNase 1 and RNase A, in order to eliminate signal due to exogenous nucleic acid - PicoGreen dye binds poorly to small oligonucleotides. At this point, Ad5 DNA is enclosed within a protein capsid, and is resistant to DNase. The sample is then treated with a combination of SDS and EDTA. This combination effectively inhibits DNase, while SDS lyses the virus, freeing Ad5 DNA. Samples are then applied to a microtiter plate, PicoGreen dye is added, and the plate is read on a fluorescent plate reader (settings of ex: 485 nm, em: 538 nm). Resulting fluorescence is fitted to a standard curve of bacteriophage λ DNA to determine Ad5 DNA concentration (λ and Ad5 DNA give equivalent signal), and concentration is converted to Ad5 particles/ml using the conversion factor: 1 μ g Ad5 DNA = 2.5×10^{10} Ad5 particles. Samples can also be analyzed without nuclease pretreatment, which will give total nucleic acid (exogenous nucleic acid + Ad5 DNA). This can be compared with the result using nuclease pretreatment, in order to assess sample purity.

Assay

This assay is based on the PicoGreen dsDNA Quantitation Kit (Molecular Probes, Cat.# P-7589)

Standard preparation

Bacteriophage λ DNA is used as the standard (supplied with the kit, at 100 μ g/mL). Perform an initial 1:20 dilution to 5000 ng/ml - 10 μ l into 190 μ l of 0.1% SDS, 25mM EDTA, then five serial 1:3 dilutions - 50 μ l into 100 μ l of 0.1% SDS, 25mM EDTA - to give a six point standard series with the range 21-5000 ng/ml. A blank, consisting of 0.1% SDS, 25mM EDTA is run with the assay.

Sample preparation

Sample dilutions: typically, several (two to three) dilution levels for each sample are run in the assay, and the results are averaged. Crude (harvest) sample typically need to be diluted in the range of 1:2 to 1:16 (with nuclease pretreatment) and 1:4 to 1:32 (without nuclease pretreatment); while purified samples are run undiluted to diluted 1:8.

DNase/RNase treatment:

To 10 μ l of appropriately diluted sample - also prepare a blank, consisting of 10mM Tris, pH 7.5, 1mM EDTA (TE) instead of sample, add 10 μ l DNase/RNase mix, consisting of a 1:1:8 (v:v:v) mix of:

DNase 1 (Boehringer Mannheim, at 10 U/ μ l)

RNase A (Boehringer Mannheim, at 10 mg/ml)

2X DNase buffer (80mM Tris, pH 7.9, 20mM NaCl, 12mM MgCl₂)

Incubate at 37 C for 15 min.

DNase inhibition/virus lysis:

Dilute samples two fold in 50mM EDTA/0.2% SDS. For nuclease pretreated samples, add 20 μ l; for untreated samples, add 15 μ l to 15 μ l of appropriately diluted sample. Incubate at 37 C for 15 minutes. After incubation, briefly centrifuge samples to bring down any condensate from sides/top of tubes.

PicoGreen assay

Add 10 μ l of standards, samples and blanks (usually run in duplicate) to 96 well plate, then add 190 μ l of PicoGreen reagent (stock reagent diluted 380 fold in TE, make up just prior to use and store in the dark). Incubate in the dark at room temperature for 3-5 minutes, read plate on fluorescent plate reader, Molecular Devices fmax settings of ex: 485 nm, em: 538 nm.

Data analysis

Data is analyzed using Softmax Pro software (see manuals for detailed instructions). The standard blank is used to correct standard and non-nuclease pretreated sample readings, while the DNase/RNase blank is used to correct nuclease pretreated sample readings. A quadratic curve fit is used. Readings outside the standard curve are not used in final results.