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GB/T 34796-2017

Quantification and purity analysis of nucleic acid concentration in solution - Ultraviolet spectrophotometry

水溶液中核酸的浓度和纯度检测 紫外分光光度法

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1 Scope

China's national method for measuring the concentration and purity of nucleic acids in solution by ultraviolet spectrophotometry. It specifies the principle, the reagents and materials, the instruments, the sample preparation, and the determination of concentration and purity by absorbance. It is the first measurement made on almost every DNA or RNA preparation in every molecular biology laboratory, and it works because nucleic acid bases absorb strongly at 260 nanometres. Concentration follows directly from the absorbance there. Purity comes from ratios: the absorbance at 260 against that at 280 says how much protein is carried along, and against that at 230 says how much of the extraction chemistry - guanidine, phenol, chaotropic salts - is left behind. Those ratios matter because the contaminants inhibit the enzymes used downstream, and a preparation that reads a good concentration but a poor ratio will fail in a PCR or a sequencing library for reasons that look like something else entirely.

This Standard specifies the principle, sample preparation, and detection method for detecting the concentration and purity of nucleic acids in aqueous solutions using ultraviolet spectrophotometry. This Standard applies to the detection of nucleic acid concentration and purity in aqueous solutions, as well as to the quantitative determination and purity analysis of nucleic acid samples in aqueous solutions with concentrations of 5 ng/μL and above.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited

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applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

GB/T 6682, Water for analytical laboratory use - Specification and test methods GB/T 11165, pH meter

GB/T 26813, Double beam UV/VIS spectrophotometer JJG 646, Verification regulation of locomotive pipette

3 Abbreviated terms

For the purposes of this document, the following abbreviated terms apply. DNA. deoxyribonucleic acid RNA. ribonucleic acid

4 Principle

Nucleic acids contain conjugated double bonds between purine and pyrimidine bases, which have the property of absorbing ultraviolet light, with the maximum absorption value at 250 nm ~ 270 nm. After the base forms a nucleotide with pentose and phosphate, its maximum absorption peak does not change. The maximum absorption wavelength is about 260 nm, and the absorption low peak is at 230 nm. At a wavelength of 260 nm, the optical density of 1 unit OD value is equivalent to a concentration of 50 μg/mL for double-stranded DNA, 40 μg/mL for single-stranded DNA and RNA, and 33 μg/mL for single-stranded oligonucleotides. This quantity-concentration relationship can be used to calculate the concentration of nucleic acid samples.

5 Reagents and materials

Unless otherwise specified, all reagents used in this method shall be analytical reagents, and the water shall be Grade-I water as specified in GB/T 6682.

5.1 DNA standard solution Calf thymus DNA standard solution.

5.2 Tris-hydroxymethylaminomethane buffer (1 mol/L Tris-HCl, pH 7.4) Weigh 121.1 g of Tris into a 1 L beaker; add 800 mL of deionized water; stir thoroughly to dissolve; use NaOH to adjust the pH; bring the volume to 1 L; dispense; autoclave at 103 kPa (

1.05 kg/cm2) for 20 min; store at room temperature for later use.

5.3 EDTA (500 mmol/L, pH 8.0) Weigh 186.1 g of Na2EDTA·2H2O; place it in a 1 L beaker; add about 800 mL of deionized water; stir thoroughly; use NaOH to adjust the pH to 8.0 (EDTA can only be completely dissolved at pH 8.0); fix the volume to 1 L; dispense into containers; autoclave at 103 kPa (

1.05 kg/cm2) for 20 minutes; store at room temperature for later use.

5.4 TE buffer (10xTE Buffer, pH 7.4) Measure 100 mL of 1 mol/L Tris-HCl (pH 7.4) and 20

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mL of 500 mmol/L EDTA (pH 8.0) into a 1 L beaker; add 800 mL of deionized water; mix well; bring the volume to 1 L; after dispensing, autoclave at 103 kPa (

1.05 kg/cm2) for 20 min; store at room temperature for later use.

6 Main instruments and equipment

6.1 Ultraviolet spectrophotometer or nucleic acid protein analyzer, which shall meet the requirements of GB/T 26813.

6.2 Vortex generator.

6.3 Electronic balance. accuracy,

0.1 mg and

0.01 mg, respectively.

6.4 pH meter, grade 0.1, which shall comply with the requirements of GB/T 11165.

6.5 Adjustable pipettes. 0.5 μL ~ 10 μL, 10 μL ~ 100 μL, 100 μL ~ 1 000 μL, which shall comply with the requirements of JJG 646.

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7 Sample preparation

Dissolve an appropriate amount of nucleic acid sample in sterile water or TE solution; dilute it to the working curve range for measurement.

8.1 Instrument setup conditions and procedures

8.1.1 Nucleic acid concentration detection Detection wavelength. 260 nm.

8.1.2 Nucleic acid purity detection Detection wavelengths. 260 nm, 230 nm, 280 nm.

8.2 Determination Take an appropriate amount of nucleic acid solution (solution volume depending on the instrument requirements) for detection; use diluted solution to zero; calculate the concentrations of DNA double strand, DNA single strand, and RNA based on the absorbance of nucleic acid at wavelengths of 260 nm, 230 nm, and 280 nm; determine the purity of nucleic acid based on the OD260/OD280 and OD260/OD230 ratios. Repeat the measurement three times and take the average value. The properties of nucleoside triphosphates are shown in Table A.1, and the spectrophotometric results of purified DNA are shown in Table B.1.

8.3 Result calculation

8.3.1 Calculation of nucleic acid concentration detection results Calculate nucleic acid concentration using the following formula.

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