OLIGONUCLEOTIDE EXTINCTION COEFFICIENT KIT

ProductInformation

Bulletin No. MB-310
For Products No. EXT-1-50 and EXT-1-200
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INTRODUCTION

The oligonucleotide extinction coefficient kit is designed to accurately measure oligonucleotide extinction coefficients. The method relies upon the measurement of the hyperchromicity resulting from exhaustive hydrolysis of an oligonucleotide to mononucleotides[1]. For single stranded oligonucleotides (SSO), exhaustive hydrolysis is accomplished using the 3' to 5' phosphodiesterase activity of snake venom phosphodiesterase (SVP) [2]. For double stranded oligonucleotides (DSOs), and SSOs containing double stranded regions, the exonuclease and endonuclease activities of SVP and DNase I (not included) can be combined to rapidly hydrolyze these molecules to mononucleotides. After exhaustive hydrolysis the oligonucleotide extinction coefficient (ε_{ODN}) is given by:

$$\varepsilon_{ODN}=H \times \varepsilon_{sum} \qquad \text{eq. 1.}$$

H is the ratio of the prehydrolysis absorbance to the post hydrolysis absorbance (i.e. $H=A_0/A_{\square}$) and $\varepsilon_{sum}=n_A\varepsilon_A+n_C\varepsilon_C+n_G\varepsilon_G+n_T\varepsilon_T$. n_i and ε_i are the number and extinction coefficient respectively of nucleotide i in the oligonucleotide.

Three common calculations are used to estimate the 260 nm extinction coefficient of an SSO. Method 1 assumes that an SSO at 33 µg/ml will have an absorbance of one[3]. The second method assumes ε_{ODN} is the sum of the individual nucleotide extinction coefficients[3,4]. That is, $\varepsilon_{ODN}=n_A\varepsilon_A+n_C\varepsilon_C+n_G\varepsilon_G+n_T\varepsilon_T$. The third method accounts for base composition and sequence by calculating the extinction coefficient as: $\varepsilon_{ODN}=\varepsilon_1+\varepsilon_m+2\square\varepsilon_{i,i+1}-\square\varepsilon_i$, where $\varepsilon_{i,i+1}$ are the dinucleotide extinction coefficients, ε_i are as above and ε_1 and ε_m are the first and last mononucleotide extinction coefficients[5]. Double stranded oligonucleotide (DSO) extinction coefficients are commonly calculated assuming that a 50 µg/ml solution will have an absorbance of one[3]. In addition, extinction coefficients can be calculated by multiplying the sum from Method 2 by a factor which attempts to account for the base stacking induced hypochromicity[6]. For a series of SSO's and DSO's it was reported that the extinction coefficients measured by exhaustive hydrolysis were within 20% of the calculated values[1].

ITEMS INCLUDED IN THE KIT
Sufficient for 50 or 200 determinations respectively

	EXT-1-50	EXT-1-200
Snake Venom Phosdiesterase (SVP) from *Crotalus durissus terrificus*, Catalog No. P 5785 (solution in 50% glycerol containing 5 mM Tris HCl, pH 7.5). One unit will hydrolyze 1.0 µmole of bis(p-nitrophenyl)phosphate per minute at pH 8.9 at 25°C.	200 µl	800 µl
20X Hydrolysis Buffer, Catalog No. H 6655 (2 M Tris HCl pH 9.1, 0.2 M $MgCl_2$)	1.5 ml	4 x 1.5 ml
Thymidine 5'-monophosphate p-nitrophenyl ester, Catalog No. T 4510	5 mg	2 x 5 mg

ITEMS NOT INCLUDED IN THE KIT (OPTIONAL)

Primer, pBR322, *Hind*III, counterclockwise (for single stranded control, ε_{sum}=178.5 $mM^{-1}cm^{-1}$, ε_{ODN}=150□3 $mM^{-1}cm^{-1}$ [a]), Catalog No. P 6427
DNase I from bovine pancreas (for double stranded extinction coefficients), Catalog No. D 7291
One unit will cause a ΔA_{260} of 0.001 per minute per ml reaction mixture using calf thymus DNA as substrate.

PRECAUTIONS

Sigma=s Oligonucleotide Extinction Coefficient Kit is for laboratory use only; not for drug, household or other uses. Kit contains components which are hazardous. Warning statements are included on the label or in the components section of this bulletin where applicable. Since SVP is isolated from snake venom it is recommended that gloves be worn at all times.

STORAGE

Store all kit components at □20□C.

PREPARATION OF SOLUTIONS

For 1X hydrolysis buffer add 19 volumes of H_2O to 1 volume of 20X buffer. The 20X hydrolysis buffer may develop a precipitate on storage. If this happens, warm the buffer at 37□C to dissolve the precipitate.
To the 5 mg of thymidine 5'-monophosphate p-nitrophenyl ester, add 220 µl of H_2O. For extended storage, the solution may be frozen in aliquots.

PROCEDURE

1. Zero two matched semimicro cuvettes at 260 nm containing 200 µl of 1X hydrolysis buffer (0.1 M Tris HCL pH 8.9, 10 mM $MgCl_2$). Empty and dry the sample cuvette.

2. To the sample cuvette add the oligomer (A_0 between 0.1 and 0.3) in 200 µl of 1X buffer and record the absorbance as A_0. Record the amount the oligonucleotide was diluted (i.e. 5 µl to 200 µl or 40X). This dilution should be used to calculate the concentration of the bulk or parent oligonucleotide solution[b].

3. Add 2 µl of SVP to the sample and blank cuvettes[c,d] and monitor the increase in absorbance for a minimum of five minutes after the hydrolysis appears to be complete. Transfer 100 µl of the reaction to a microcentrifuge tube containing 2 µl of the thymidine 5'-monophosphate p-nitrophenyl ester solution. The almost immediate appearance of p-nitrophenoxide (yellow) ensures that there was still SVP activity present and the measured hyperchromic shift was due to substrate exhaustion[e]. Record the absorbance as $A_{\square}$.

4. Calculate H ($H=A_0/A_{\square}$).

5. For greatest accuracy, repeat steps 2-4 at two additional A_0's to verify that H is concentration independent and reproducible.

6. Calculate ε_{sum} according to $\varepsilon_{sum}=n_A\varepsilon_A+n_C\varepsilon_C+n_G\varepsilon_G+n_T\varepsilon_T$ where:

n_A=number of A's	ε_A=15.4$mM^{-1}cm^{-1}$
n_C=number of C's	ε_C= 7.4$mM^{-1}cm^{-1}$
n_G=number of G's	ε_G=11.5$mM^{-1}cm^{-1}$
n_T=number of T's	ε_T= 8.7$mM^{-1}cm^{-1}$

7. If H is concentration independent, calculate ε_{ODN} according to:

$$\varepsilon_{ODN}=H_{avg} \times \varepsilon_{sum}$$

where H_{avg} is the average H obtained from the three separate determinations.

Notes

a. The extinction coefficient was measured as described for two lots of pBR322 Primer (*Hind*III), counterclockwise, Catalog No. P 6427, on Shimadzu (UV160U) and a Beckman (DU-50) spectrophotometers. The extinction coefficients determined using two Shimadzu spectro-photometers were each 151 □2 $mM^{-1}cm^{-1}$. Using the Beckman instrument the extinction coefficient was 147□3 $mM^{-1}cm^{-1}$.

b. Since it is possible that the conformation of the oligonucleotide will be dependent upon buffer composition, the concentration of the oligonucleotide should be measured under the same conditions as used for the extinction coefficient measurement.

c. The number of determinations possible using this kit may be extended by adding the enzyme only to the sample cuvette. In this case, the hyperchromicity should be corrected by subtracting the

absorbance due to SVP (typically □0.005). The 260 nm SVP absorbance should be independently measured (i.e. in the absence of an oligonucleotide) using conditions that are identical to those used for the hydrolysis reactions. For statistical accuracy, it is recommended that the enzyme=s absorbance be initially measured several times. During subsequent extinction coefficient determinations it is desirable to verify the enzyme=s absorbance.

d. For DSOs and SSOs that are reluctantly hydrolyzed by SVP alone, 200 units of bovine pancreatic DNase I is also added to the sample and blank cuvettes.

e. Hydrolysis completion can be confirmed by assaying a 5' end-labeled reaction product for oligomers as described by Kallansrud and Ward[1].

References

1. Kallansrud,G., and Ward, B. Anal. Biochem. **236**, 134-138 (1996)
2. Cantor, C.R., and Tinoco, I. J. Mol. Biol. **13**, 65-77 (1965)
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