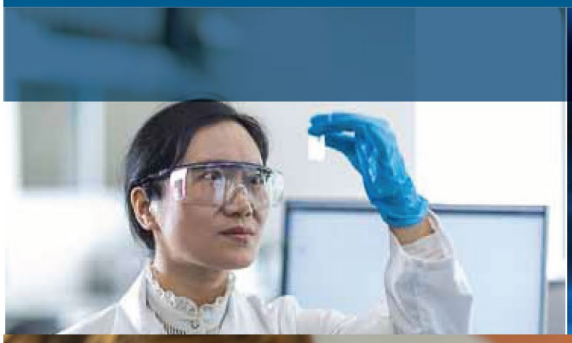
Agilent

Trusted Answers

DNA Thermal Melts Simulation

Student instructions



Thermal melts (replication) experiment

Student instructions- In lab

Introduction

This experiment requires you know how to:

- Use a UV-Vis spectrophotometer and handle cuvettes

You should also have a basic understanding of:

- The principles of UV-Vis spectroscopy. For a refresher on UV-Vis spectroscopy, see the Agilent UV-Vis Spectroscopy Primer.

- DNA thermal melt principles and measurement.

In this experiment, you will:

- Use a UV-Vis to record UV-Vis spectra

- Use the spectra to determine a simulated DNA melting point

Theory

In this experiment, you will measure a provided sample that mimics the reaction of DNA as it is slowly heated. DNA, when exposed to heat or variations of pH, can transform from its characteristic double stranded helix structure to single strands. This is due to the breaking of the hydrogen bonding between the base pairs making up the DNA.

Nucleic acids absorb strongly in the UV region, making them ideal for study by UV spectroscopy. Typically, a strong absorbance band is found around 260 nm. DNA is made up of chains containing the four bases, adenine (A), cytosine (C), guanine (G), and thymine (T). These are paired across the strands in pairs of A-C and G-T. The proportions of these base pairs in a pure DNA molecule can be determined due to the observation that the G-C bonds are stronger than the A-C bonds. This means more energy (a higher temperature) will be required to denature (or melt) the DNA strand, if the proportion of G-C bonds is higher.

At the melting point, the absorbance profile of the DNA changes as the two strands break apart. As the strands being to separate, the absorbance of the solution increases until the strands completely separate, and at which point the absorbance plateaus. The melting point, defined as the temperature where half the DNA exists as single strands, is called the thermal melt temperature, T_m. The T_m value can be compared with known DNA composition to indicate the proportion of G-C bonds in the DNA.

Once the DNA strands are separated, cooling the sample will cause the DNA strands to re-combine. This can cause hybridization. Measuring the T_m response of a DNA molecule that has been heated and cooled can indicate how stable, or resistant the DNA is to thermal influences.

In this experiment, you will be using a UV-Vis spectrometer to measure a sample of approximately 10% N-isopropylacrylamide solution in distilled water. This compound has similar observed properties when heated as DNA. In the case of this compound, the observed wavelength is different, 450 nm (compared to 260 nm for real DNA). You will use temperature control to precisely increase the temperature over time and record Absorbance vs Temperature.

From the data collected you will calculate the T_m for this compound. A useful reference for Thermal Melt experiments and ramp rates is provided by Agilent.

Apparatus

You will need:

- Approximately 10% N-isopropylacrylamide solution in distilled water. This may have been supplied in a sealed cuvette.
- Two UV-Vis glass cuvettes, with caps. (One if sample is supplied in a sealed cuvette)
- Distilled water in reagent bottle, (for rinsing and dilution)
- A UV-Vis spectrophotometer with the ability to perform and record absorbance measurements over a temperature range
- Spreadsheet/graphing software

1. Method

1.1. Rinse cuvettes with distilled water and ensure that the measuring surfaces are clean.

1.2. Fill one cuvette with water and the other with the 10% N-isopropylacrylamide solution.

1.3. Using the supplied solution and the distilled water as a reference follow the manufacturer of the UV-Vis spectrometers instructions to perform a baseline measurement, then using the prepared sample, measure a Temperature Vs Absorbance experiment using the parameters in Table 1.

Report: Thermal melts (replication) experiment

Name:
_____

Date: Time:_____
_____

Introduction

What are you doing and why?

Methods

Write a summary of the method you used to determine the Tm value of the solution.

Results

Attach the results section with your report.

Discussion

Include discussion of: Any difference observed between the three calculations of the Tm values. Given that the second run used the same sample at a faster temperature ramp rate explain any observed differences.

Conclusion

Describe what the data you collected tells you. How would this experiment be modified for measurement of T_m values for DNA samples? What key parameters and method adjustments would need to be made?

Agilent

Trusted Answers

DNA Thermal Melts Simulation

Demonstrator instructions

Apparatus and solutions required:

- Approximately 10% N-isopropylacrylamide solution in distilled water. For convenience, the sample may be supplied in a sealed cuvette. It is recommended each available UV-Vis system is supplied with a sample for each group to use.

  Poly-N-isopropylacrylamide with the following properties is recommended:

  - Phase transition hydrogel
  - An number average molecular weight (M_n) of 10,000-30,000
  -Sigma-Aldrich Product #535311 is recommended.

  This compound dissolves in water and is stable over long periods of time. The compound in water produces a 100% reversible thermal melt curve. Both heating and cooling produces repeatable results.

  When performing a thermal melt analysis, a smooth curve will be obtained with a well-defined T_m at 35.6 ºC.

  At temperatures below T_m the solution is clear. Above the T_m, a milky-cloudy scattering colloid is observed.

- Two UV-Vis glass cuvettes (one if sample is supplied in a sealed cuvette). The second cuvette is to be used for the reference measurement, or in the reference position if using a double beam system. The reference solution should be distilled water.

- Distilled water in reagent bottle (for rinsing and dilution)

- A UV-Vis spectrophotometer. The system should be set up for Thermal melt measurements using a standard 10 mm pathlength cuvette. The demonstrator should be able to demonstrate the use of the software, accessory setup and method and date collection.

- Graph paper, ruler and pencil. (not required if calibration curve is to be plotted electronically)

Student prerequisites:

Before the session review the prerequisites with the cohort.

The students should be familiar with or understand:

- Safe working practices in the laboratory

- Use of a UV-Vis spectrophotometer and cuvette handling

They should also have a basic understanding of:

- The principles of UV-Vis spectroscopy. For a refresher on UV-Vis spectroscopy, see the Agilent UV-Vis Spectroscopy Primer.

- DNA thermal melt principles and measurement.

General notes and guidance

- Students can perform the work individually or in pairs. All calculations should be individual work.

Script

This script follows a version of the student practical for online delivery.

Introduction

1. In this experiment, I will demonstrate a DNA thermal melt measurement using a sample that mimics the reaction of DNA as it is slowly heated. The sample used will be Poly-N-isopropylacrylamide which is a phase transition hydrogel. You should understand the theory of performing DNA melt measurements and keep in mind the result obtained are not reflective of those expected for DNA samples. Action: Show the clear Poly-N-isopropylacrylamide solution at ambient temperature. Optional: Show an already warmed solution to show it turns clouding above the "Tm".

2. In this experiment we will be using a UV-Vis Spectrophotometer to monitor the transmission of the solution as it is heated. To perform these measurements specialized accessories are required to slowly and precisely raise, and monitor, the temperature of the solution. This will allow us to record and the Absorbance vs Temperature plot of the solution at a fixed wavelength. Action: Point out the temperature control accessory, if visible, and describe how the samples are heated. Typically this will be either by use of peltier temperature control at the cuvette, or with a water bath which circulates heated water through the cuvette holder.

3. Once a measurement of the Absorbance vs Temperature of the solution has be undertaken the data collected will be used to calculate the Tm value of the samples. This will be performed by you using two manual methods and if available using the UV-Vis spectrometer software.

Method

4. I will start by using the UV-Vis software control to set the parameters for the measurement. In this experiment we are using a fixed wavelength and taking absorbance measurements at a temperature interval of 0.5°C. The measurements will start at 25 °C and finish when the sample reaches 50 °C. Action: Open the UV-Vis software control software and show how to navigate to the method parameter for a thermal melt measurement. Enter the parameters as per Table 1. Comment on whether stirring is to be used for the measurement.

5. (If using stirring). The magnetic stirring bead is inserted into the cuvette. The stirring bead is usually controlled either through the software or a control on the accessory. The stirring bead ensures the whole of the sample is heated uniformly. Stirring should not be so rapid as to entrain air into the sample, but needs to be adequate to mix the sample from top to bottom. Action: Show a close-up of the stirring bead and insertion into the cuvette. (Note some cuvettes may have inbuilt stirring beads).

6. I will now transfer my reference solution to a cuvette. The reference solution, in this case, is distilled water. If measuring a DNA sample, the reference would be the same solution used to prepare the DNA but omitting the DNA. I will use the reference to set a baseline (zero) measurement at the wavelength to be used. Action: Take the reference solution and following the instruction provided by your UV-Vis spectrometer supplier use it to zero the instrument at the wavelength selected.

7. The next step is to take the sample, which is at room temperature, and transfer it to a cuvette. As we are heating the sample it is good practice to ensure the cuvette is capped with a lid, or a small piece of plastic film. This will prevent evaporation of the sample. The cuvette should be handled carefully to prevent scratching or marking of the optical surfaces. Any marks, or stray fingerprints, should be wiped off the cuvette with a soft cloth. I'll insert the sample cuvette into the sample holder. The measurement is activated through the software control. Action: Transfer the sample to a cuvette and demonstrate capping and proper sample handing. You could point