Determination of Enzyme Thermal Stability

The thermal stability of the enzyme was evaluated using a fluorescence dye-based Protein Thermal Shift (PTS) assay. This method monitors the changes in fluorescence intensity caused by the binding of a hydrophobic dye to the hydrophobic regions of proteins that become exposed during thermal denaturation. The experiments were conducted on an Applied Biosystems real-time PCR instrument (Thermo Fisher Scientific), with excitation and emission wavelengths set to 520 ± 10 nm and 558 ± 12 nm, respectively.

For each assay, 5 μg of purified enzyme was mixed with 2.5 μL of 8 × Protein Thermal Shift dye. Subsequently, 5 μL of reaction buffer was added, and the mixture was adjusted to a final volume of 20 μL with phosphate buffer (50 mM Na2HPO4, 100 mM NaCl, pH 7.5). The prepared reaction mixture was transferred into the PCR instrument, equilibrated at 25 °C for 2 min, and then subjected to a temperature gradient from 25 °C to 99 °C with a ramp rate of 0.05 °C/min, while fluorescence was recorded in real time.

The fluorescence intensity profile reflected the thermal unfolding process of the protein. The melting temperature (Tm) was determined from the first derivative of the fluorescence versus temperature curve and used as a quantitative measure of enzyme thermal stability.