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PR No. 1000026324 (Rfx No. 6100001165)

Detailed Technical Specifications for Isothermal Calorimeter ITC:

- 1. This system is utilized for measuring heat released or absorbed during a binding interaction, characterization of molecular interactions, determination of thermodynamic parameters such as K_d, ΔH, ΔS, stoichiometry of binding etc for interactions of ligands with biomolecules such as DNA, RNA,lipids etc The fully automated system must be equipped with control unit, wash module, autopipetting syringe with tower filling and assemblies, injection syringes and other start up accessories for full functional of the ITC, necessary software for instrument control, operation, data analysis and viewing. For all the aforesaid molecular interactions, the measurements needs to be directly measured values of heat change during the ligand-biomolecular interactions through a heat compensation power feedback loop. For this system, the feedback loop is highly sensitive with a detection sensitivity of 50 nanocalorie.
- 2. The material of the cell must be made of inert material and highly resistant to extreme pH conditions, organic solvents etc. The cell should have an attached Peltier element with fast equilibration and high sensitivity towards heat change. The response time needs to be quick (~≤ 8 seconds) to avoid data loss. We work with RNA molecules and at times, some low yielding protein molecules which is synthesized in lab and thus we require cell with low working volume (cell volume plus dead volume <300µl). In order to have a homogeneous mixing, the cell must have a stiring speed of 1-1000 rpm or more. Since we also want to perform binding studies for sensitive biomolecules, the system should be able to do a thorough cleaning.
- 3. The injection syringe volume must be less than or equal to 60 μl with minimum injection volume of ≤1.0μl and with a high precision is required. The feedback loop should also come with multiple feedback options for performing a plethora of binding/interaction processes.
- 4. The system should also have a less equilibration time (≤ 6 min) or lower between 25°C to 5°C. The operating temperature range is in the range 2° to 80°C with temperature stability $\sim \pm 0.0001$ °C at 25°C.
- 5. The system should be able to detect molecular interactions with binding constants ranging from sub-millimolar (10² M⁻¹) to nanomolar range (10⁹ M⁻¹) (for normal binding) and 10² to 10¹² M⁻¹ (for competitive binding).
- 6. The system should be automated and controlled via instrument software and should have all the necessary accessories required for proper functioning such as automated washing and cleaning of cell and syringe, auto-pipetting syringe and assemblies, injection syringes, purging options to remove air bubbles.
- 7. The system should have a **compatible software** with the latest model and should be capable of running the instrument, injector control, sample loading injector, and providing suitable **binding models** such as

- single site, two site, sequential site, competitive site and enzyme kinetics. Non-linear least square analysis of the data should include calculations to rectify the excluded concentrations of biomolecules and ligands during each injection. The data obtained should be easy to export and use in any other format and should be of high resolution for publication purpose.
- 8. The necessary software required for the instrument should be provided. The analysis software should provide copies of offline analysis software and should not require a separate software supporting license.
- 9. Other accessories for the instrument should be provided such as extra tubings, syringe wash model, filling port adapter with needle, bottle kits, bottling tubing external. Additional injection syringe must be provided as well.
- 10. The system must come with a standard 1-year warranty.