BOSE INSTITUTE
Centenary Building,
P-1/12, CIT Scheme – VII M, Kankurgachi,
Kolkata – 700 054 (INDIA)

Minutes of the Pre-bid Conference held on 16.9.2020 at 1.00 p.m. in the Seminar room of the Department of Biochemistry at centenary building of the Institute regarding Tender Notice No. BI-K/E-TEND/03/2020-21 with tender id : 2020_BIK_582587_1 for procurement of Size Exclusion Chromatography Multi Angle Light Scattering MALS and Dynamic Light Scattering Detector DLS with High Performance Liquid Chromatography

Members present :

Dr. Jayanta Mukhopadhyay (Online)
Dr.Abhrajyoti Ghosh
Dr. Smarajit Polley

Bidders present ::

- Representative of Galaxy International Corporation, Hyderabad
- Representative of Spinco Biotech
- Representative of Agilent (online)

Technical Specifications for Size Exclusion Chromatography-Multi Angle Light Scattering (MALS) & Dynamic Light Scattering Detector (DLS) With High Performance Liquid Chromatography

Integrated Multi Angle Static & Dynamic Light Scattering (SLS/DLS) with a Differential Refractive Index Detector for characterization of proteins and other biopolymers through *absolute* molecular weight determination under Size Exclusion Chromatography-Multi Angle Light Scattering Detector (SEC-MALS/DLS)/Gel Filtration Chromatography-Multi Angle Light Scattering mode (GFC-MALS/DLS) is to be quoted. The system shall be compatible with conventional HPLC systems/Fast Protein Liquid Chromatography (FPLC) systems, for online characterization. The DLS system shall be suitable for conducting *simultaneous* Static and Dynamic Light Scattering Detection under chromatography, using the same flow cell besides functioning as a batch system. The offered system shall be under a Windows-PC based software workstation for collection and processing of data. Quoted models shall have at least 5 user references in India.

I. SLS System Specifications

Light Source	658 +/- 30 nm Laser with a power control, programmable in the range 10% - 100%. The system shall have rear laser monitor for stable laser output and also a forward transmission monitor to correct signals for absorbing samples.
Detectors	Minimum 7 angles, within the range 15 - 160 Degrees, with simultaneous measurement capability using PIN diodes. The detector

	shall have dedicated ***In-situ*** cleaning system based on Radio Frequency Ultrasonic Generator to minimize particulate adhesion to the flow cell windows.
Detector Resolution	24 bit
Operating Temperatures	Ambient
Molar Mass Range	<10e3 to 10e7 Da or better
Molecular Size Range	10 - 200 nm or more as RMS radius
Sensitivity	0.50 micrograms BSA or better under HPLC-SEC conditions;
Measurement Options	Online-Mode (Chromatography) and Batch Mode (with volumes 1-2 ml). Provision should also be available for measurements in the batch mode using 10-20 microliter sample volumes.
Software	Should report number, weight, Z-average molecular weights and root mean square sizes and their distributions. Shall be able to report the standard deviations of the measurements. Software should correct for the band broadening effects arising from interfacing light scattering detectors to HPLC systems with additional detectors such as UV-VIS and also report standard deviations of the measurements.
Solvent Compatibility	All-solvent compatible (aqueous and organic)
Computer Interface	Ethernet

II. DLS System

Should be fully integrated with the SLS System for both online and off-line measurements of the molecular size information and shall use the same flow cell of MALS for measurements.

Size Range (R_h)	1 nm to 300 nm in flow mode; Up to 1000 nm in Batch Mode with capability to determine the size distributions.
Detector	Photon-counting avalanche photo diode with an optical fiber that should Integrate with the read head of the Multi Angle SLS detector for simultaneous measurements of SLS/DLS characteristics.
Correlator	Built-in, 512 channel autocorrelator with multi-tau distribution.
Software	Should be capable of doing regularization analysis of unfractionated DLS data to retrieve underlying hydrodynamic radius distributions. The Software should also support cumulant mode of data fitting routine besides regularization. Export facility for sending results to ASCII files for processing in spreadsheet formats should be provided.

III. Refractive Index Detector (dRI)

Should be capable of being used as a concentration detector for on-line (chromatography mode) along with SLS detector for measurement of absolute molecular weights. The dRI instrument must be capable of being used off-line (batch mode via sample injection into the flow cell by means of a pump) to measure *dn/dc* of the sample at the same wavelength as that of the MALS instrument. The refractive

index detector shall be controlled by the same software as that of the SLS system and shall have the following specifications:

Light Source	:	658 +/- 30 nm
Differential Refractive Range(µRIU)	:	±4700 µRIU
Peak to Peak Noise (RIU)	:	+/- 2.5 x 10e-9
Flow Cell Volume (inlet tubing+flow cell)	:	<15 microlitres
Dynamic Range	:	22+ bits, via digital communication
Temperature Settings	:	Ambient to 50 Deg C or better
Absolute Refractive Index Range	:	1.2 – 1.8 RIU (sensitivity of +/- 0.002 RIU)
Digital Communication	:	Ethernet
Safety Sensors	:	Vapor and Liquid (leak)
Computer Interface	:	Ethernet

IV. High Performance Liquid Chromatograph

Fully Integrated Quaternary Gradient HPLC System with Auto sampler, UV Detector, Column Oven, Chromatography Software should be offered as per the below specifications. The quoted configuration should be compatible with Multi Angle Light scattering for conducting SEC-MALS studies	
Solvent Delivery Unit	• It should be a Quaternary Low-Pressure Gradient pump with Parallel-type Double Plunger design • The flow rate should be set between 0.001 to 10 mL/min • Flow rate accuracy should be less than ±2% • Flow rate precision should be less than ±0.06% RSD • The maximum pressure setting range should be 5000 Psi or above • The Gradient/concentration accuracy should be below 0.5% & Gradient/concentration precision below 0.1% RSD • Gradient curve setting function and Leak Sensor should be available
Degassing Unit	• Degassing unit should have 4 flow lines & membrane-type online degassing
Auto-Sampler	• Sample injection volume should be variable between 0.4 µL to 100 µL • Injection volume accuracy must be below ±1% • injection Precision : less than 0.25% RSD • Sample Cooler Temperature: 4 to 45 Deg C • Sample Vial Capacity : 150 or above for 1.5 mL • It should have safety features like leak sensor and automatic rack and vial recognition.
Columns	• Suitable for Size Exclusion/Gel Filtration Chromatography • Column material: suitable for proteins and other biological macromolecules, hydrophilic, suitable for aqueous solvents (pH range 4-7.5 or better) • Particle diameter: less than or equal to 5µm • I.D.: greater than 5µm and less than 10µm • Length of the column: 500mm With these common specifications as above, columns of two different pore sizes should be supplied as described below: • Pore size: a) less than or equal to 150Angstrom (2 columns); b) less than or equal to 300Angstrom (2 columns) • 2 protective guard columns for each type of column mentioned above (total 4)

	should be supplied
Column Oven	• Column oven should be forced air circulation type for uniform temperature distribution. • The temperature should be in the range Ambient to 90 Deg C in steps of 1 Deg C • Temperature stability should be 0.8 Deg C • Temperature control precision should be 0.5Deg C or better • The oven compartment should accommodate three columns
Detector	<u>Dual Wavelength UV-VIS Detector</u> • Spectral bandwidth : 8 nm • Wavelength range : 190 nm to 700 nm, • The flow cell must be temperature controlled • Wavelength accuracy : ± 1 nm • Wavelength reproducibility : ± 0.1 nm • Drift : ≤ 1 x 10^{-4} AU/Hour • Noise level : ±2.5 x10^{-6} AU • Linearity : 2.5AU (ASTM method) • Should be capable of simultaneous monitoring of two wavelengths
Data management System	Chromatography Software : Operation of the system should be via a 64 bit Windows 10 based software
Service, Warranty and Training	Complete support for equipment for at least a period of 36 months Warranty for the entire system. Same Vendor should provide onsite operator training on the system start-up, usage, maintenance, quality control, trouble-shooting with respect to the HPLC as well as the Static Light Scattering Detector.

Resolution of the Pre-bid Conference

Existing specification	Amended specification in the relevant portion to be read as
Integrated Multi Angle Static & Dynamic Light Scattering (SLS/DLS) with a Differential Refractive Index Detector for characterization of proteins and other biopolymers through *absolute* molecular weight determination under Size Exclusion Chromatography-Multi Angle Light Scattering Detector (SEC-MALS/DLS)/Gel Filtration Chromatography-Multi Angle Light Scattering mode (GFC-MALS/DLS) is to be quoted. The system shall be compatible with conventional HPLC systems/Fast Protein Liquid Chromatography (FPLC) systems, for online characterization. The DLS system shall be suitable for conducting *simultaneous* Static and Dynamic Light Scattering Detection under chromatography, using the same flow cell besides functioning as a batch system. The offered system shall be under a Windows-PC based software workstation for collection and processing of data. Quoted models shall have at least 5 user references in India.	Independent or Integrated Multi Angle Static & Dynamic Light Scattering (SLS/DLS) with a Differential Refractive Index Detector for characterization of proteins and other biopolymers through *absolute* molecular weight determination under Size Exclusion Chromatography-Multi Angle Light Scattering Detector (SEC-MALS/DLS)/Gel Filtration Chromatography-Multi Angle Light Scattering mode (GFC-MALS/DLS) is to be quoted. The system shall be compatible with conventional HPLC systems/Fast Protein Liquid Chromatography (FPLC) systems, for online characterization. The DLS system shall be suitable for conducting *simultaneous* Static and Dynamic Light Scattering Detection under chromatography, using a batch or a flow system. The offered system shall be under a Windows-PC based software workstation for collection and processing of data. Quoted models shall have at least 5 user references in India.
Light Source 658 +/- 30 nm Laser with a power control, programmable in the range 10% - 100%. The system shall have rear laser monitor for stable laser output and also a forward transmission monitor to correct signals for absorbing samples.	Light Source 520 nm – 680 nm Laser with a power control, programmable in the range 10% - 100%. The system shall have rear laser monitor for stable laser output and also a forward transmission monitor to correct signals for absorbing samples.
Detectors Minimum 7 angles, within the range 15 – 160 Degrees, with simultaneous measurement capability using PIN diodes. The detector shall have dedicated ***In-situ*** cleaning system based on Radio Frequency Ultrasonic Generator to minimize particulate adhesion to the flow cell windows.	Detectors Minimum 7 angles, within the range 5 – 160 Degrees, with simultaneous measurement capability using PIN diodes. The detector shall preferably have dedicated ***In-situ*** cleaning system based on Radio Frequency Ultrasonic Generator to minimize particulate adhesion to the flow cell windows.

Operating Temperature Ambient	Operating Temperature ambient to 50˚C or 70˚C
Software Should report number, weight, Z-average molecular weights and root mean square sizes and their distributions. Shall be able to report the standard deviations of the measurements. Software should correct for the band broadening effects arising from interfacing light scattering detectors to HPLC systems with additional detectors such as UV-VIS and also report standard deviations of the measurements.	Software Should report number, hydro dynamic diameter (Rh), Molecular Weight (Mw), and root mean square sizes and their distributions. Shall be able to report the standard deviations of the measurements. Software should offer correction for the band broadening effects arising from interfacing light scattering detectors to HPLC systems with additional UV-VIS detector and also report standard deviations of the measurements.
DLS system Should be fully integrated with the SLS System for both online and off-line measurements of the molecular size information and shall use the same flow cell of MALS for measurements.	DLS system Should be independent or fully integrated with the SLS System for both online and off-line measurements of the molecular size information.
Size Range (Ra) 1 nm to 300 nm in flow mode; Up to 1000 nm in Batch Mode with capability to determine the size distributions.	Size Range (Ra) In independent SLS/DLS system, size range should be between ≤1 nm to 1000 nm in both flow and batch modes, while in integrated SLS/DLS system, the size range should be 1 nm to 300 nm in flow mode; and upto 1000 nm in batch mode with capability to determine the size distributions are required.
Detector Photon-counting avalanche photo diode with an optical fiber that should Integrate with the read head of the Multi Angle SLS detector for simultaneous measurements of SLS/DLS characteristics.	Detector Photon-counting avalanche photo diode (APD) with an optical fiber.
Refractive Index Detector (dRI) Should be capable of being used as a concentration detector for on-line (chromatography mode) along with SLS detector for measurement of absolute molecular weights. The dRI instrument must be capable of being	Refractive Index Detector (dRI) Should be capable of being used as a concentration detector for on-line (chromatography mode) along with SLS detector for measurement of absolute molecular weights. The dRI instrument must be capable of being used off-line (batch mode via sample injection

used off-line (batch mode via sample injection into the flow cell by means of a pump) to measure *dn/dc* of the sample at the same wavelength as that of the MALS instrument. The refractive index detector shall be controlled by the same software as that of the SLS system and shall have the following specifications:	into the flow cell) to measure dn/dc of the sample at the same wavelength as that of the MALS instrument. The refractive index detector shall be controlled by the same software as that of the SLS system and shall have the following specifications:
Light Source 658 +/- 30 nm	Light Source 520 nm – 680 nm
Differential Refractive Range(µRIU) ±4700 µRIU	Differential Refractive Range(µRIU) 4500 µRIU or better
Flow Cell Volume (inlet tubing+flow cell) <15 microlitres	Flow Cell Volume (inlet tubing+flow cell) <70 microlitres
Absolute Refractive Index Range 1.2 – 1.8 RIU (sensitivity of +/- 0.002 RIU)	Absolute Refractive Index Range 1.0 – 1.8 RIU (sensitivity of +/- 0.002 RIU)
Digital Communication Ethernet	Digital Communication Ethernet or RS232
Auto sampler Sample Vial Capacity : 150 or above for 1.5 mL	Auto sampler Sample Vial Capacity : 90 or above for 1.5 mL

REGISTRAR (OFFICIATING)