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	Procurement of Size Exclusion Chromatography Multi Angle Light Scattering MALS and Dynamic Light Scattering Detector DLS With High Performance Liquid Chromatography
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Tender Reference Number: BI-K/E-TEND/03/2020-21

Procurement of Size Exclusion Chromatography Multi

Tender Title: Angle Light Scattering MALS and Dynamic Light

Scattering Detector DLS With High Performance

Liquid Chromatography

CORRIGENDUM

AMENDMENT TO THE TECHNICAL BID

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

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.

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 nmDifferential Refractive Range(μ RIU) : $\pm 4700 \mu$ RIU Peak to Peak Noise (RIU) : $+/- 2.5 \times 10e-9$ 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	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	It should be a Quaternary Low-Pressure Gradient pump with Parallel-type Double		
Unit	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 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	Dual Wavelength UV-VIS Detector		
	Spectral bandwidth : 8 nm		
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	 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	Chromatography Software : Operation of the system should be via a 64 bit Windows 10	
management	based software	
System		
Service,	Complete support for equipment for at least a period of 36 months Warranty for the	
Warranty and	entire system. Same Vendor should provide onsite operator training on the system start-	
Training	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. system shall be compatible with conventional HPLC systems/Fast Protein Liauid 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 Refractive Index Differential Detector 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 guoted. The system shall be compatible with conventional HPLC systems/Fast Protein Liauid 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	Operating Temperature
Ambient	ambient to 50°C or 70°C
Software	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.	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	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.	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)	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.	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	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.	Photon-counting avalanche photo diode (APD) with an optical fiber.
Refractive Index Detector (dRI)	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	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 <i>dn/dc</i> 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	Light Source
658 +/- 30 nm	520 nm – 680 nm
Differential Refractive Range(µRIU)	Differential Refractive Range(µRIU)
±4700 μRIU	4500 μRIU or better
Flow Cell Volume (inlet tubing+flow cell)	Flow Cell Volume (inlet tubing+flow cell)
<15 microlitres	<70 microlitres
Absolute Refractive Index Range	Absolute Refractive Index Range
1.2 – 1.8 RIU (sensitivity of +/- 0.002 RIU)	1.0 – 1.8 RIU (sensitivity of +/- 0.002 RIU)
Digital Communication	Digital Communication
Ethernet	Ethernet or RS232
Auto sampler	Auto sampler
Sample Vial Capacity: 150 or above for 1.5 mL	Sample Vial Capacity: 90 or above for 1.5 mL

REGISTRAR (OFFICIATING)