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| – GLP –                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |  | SOP – | Page 1 of 10 |
| <b>Standard Operating Procedure SOP</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |  |       |              |
| Title: Check of Function and Specifications of the Fluorescence Detector L-7485                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |  |       |              |
| Valid since: June. 1. 2000<br>This SOP replaces the version of: New version, issue 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |       |              |
| Policy: Establishing a reliable function of the instruments<br>Responsibility: Head of Laboratory                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |  |       |              |
| <p>Data/signature      Copy received by:</p> <p>Written:                      .....      Head of Testing Facility<br/>                                                .....      Head of Laboratory<br/>                                                .....      Laboratory personnel<br/>                                                .....      GLP department</p> <p>Reviewed: .....<br/>         Approved: .....</p> <p>Total number of pages: 10 and 1 pages Test Report<br/>         References: Operating Instructions for Fluorescence Detector L-7485.<br/>                           ASTM E685-79 (1979)</p> |  |       |              |

**Check Item: Four Items**

- 1 Check of lamp display
- 2 Check of auto zero function
- 3 Check of wavelength accuracy
- 4 Check of sensitivity

**Items to be Prepared:**

1. Ion exchanged water or distilled water: About 100 mL.
2. Calipers.
3. Calibrated data processor for HPLC (Hitachi Model D-7500 Integrator or equivalent), or recorder (Hitachi Model 056 Recorder or equivalent).
4. Pump for HPLC (Hitachi Model L-7100 Pump or equivalent).
5. Analog signal cable (necessary when a data processor for HPLC except for Model D-7500 or a recorder is used).

**Preparation of Instruments:**

1. Turn on the Fluorescence Detector and warm it up for about 1 hour.
2. Turn on the data processor for HPLC and recorder. Warm them up for about 10 minutes.

## 1 Check of Lamp Display

### Content of Check:

Lamp display check.

### Check Procedure:

1. Turn on the Power switch of the Detector.
2. The screen appears on the liquid crystal display.
3. Self-diagnosis is performed.  
If abnormal, Error message is presented on the liquid crystal display. If normal, Monitor Screen appears.

### Actual Example:



FL Detector 8107064-00  
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**NOTICE:** Software version may be changed.



(Self-diagnosis check)

| TIME | Ex  | Em  | FL  | PROG | LAMP | [1]  |
|------|-----|-----|-----|------|------|------|
| 0.0  | 250 | 350 | 0.0 | 1    | Xe   | (D1) |

**NOTICE:** This screen is an example.

### Specification:

Lamp display on Monitor Screen should be {Xe}.

This means that the Xe lamp is lit normally.

### Remarks:

The following items are checked by the self-diagnosis check.

1. Memory check.
2. Function check of wavelength motor.
3. Lighting check of Xe lamp.

## 2 Check of Auto Zero Function

### Content of Check:

Auto zero function is checked.

### Check Procedure:

1. Make sure Monitor Screen (FL display) is displayed.
2. Set the excitation wavelength to 350 nm, and fluorescence wavelength to 450 nm with the **WAVE LENGTH** key.
3. Set the time constant to 2.0s with the **PARAM SET** key.
4. Press the **AUTO ZERO** key and read the FL display on Monitor Screen.

### Specification:

FL display on Monitor Screen should be -0.1 to +0.1.

**NOTICE:** When a solution that emits fluorescence of which wavelength is 450 nm with the excitation light of 350 nm is in the cell, the FL display may be out of  $\pm 0.1$  in some cases. If so, substitute distilled water for the solution in the cell, and perform measurements again.

### 3 Check of Wavelength Accuracy

#### Content of Check:

The spectral line of 436 nm emitted from a fluorescent lamp is used.

The light emitted from a fluorescent lamp is introduced into the fluorescence monochromator and the spectral line is used to check wavelength accuracy of the monochromator.

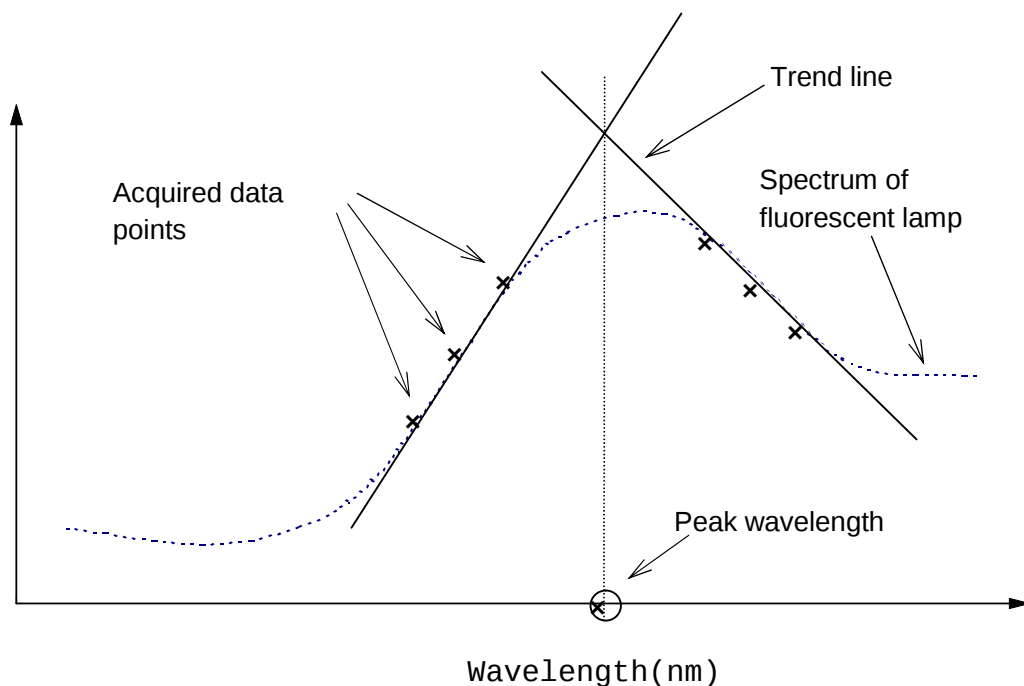
Using the emission monochromator set at the wavelength the bright line is detected, the wavelength accuracy of excitation monochromator is determined by checking the wavelength of excitation light which makes Raman scatter at 436 nm and comparing with theoretical value(380 nm).

#### Items to be Prepared:

1. Fluorescent room lamp
2. Graph sheet

#### Check Procedure:

1. Check of wavelength accuracy of fluorescence monochromator
  - 1a. Remove the light sampling screw of the flow cell unit.
  - 1b. Set PMT voltage "LOW" with the **PARAM SET** key.
  - 1c. Set Em bandwidth "STANDARD" with the **PARAM SET** key.
  - 1d. Turn off the lamp with the **PARAM SET** key.
  - 1e. Make the Display Mode be Em < M > with the **UTILITY** key.
  - 1f. Set the fluorescence wavelength (Em) to 426 nm with the **WAVE LENGTH** key, and read the energy value.
  - 1g. In the same way, read the energy values at 428nm and 430nm.
  - 1h. Plot these points on a graph sheet and confirm them in a row. If not, add another data point and acquire at least three data points in a row.
  - 1i. Draw a trend line of these points.
  - 1j. In the same way as (f) and (g), read the energy values at 442nm, 444nm and 446nm.
  - 1k. Draw another trend line of these points in the same way of (h) and (i).
  - 1l. Read the wavelength at the cross point of the two trend lines.



- NOTICE 1:** When checking the accuracy of the fluorescence monochromator, take special precautions to ensure that radiation from other sources cannot enter the instrument (e.g. do not place the unit near a window or near a source of room light).
- NOTICE 2:** If you move around in front of the sample chamber during the check of the fluorescence wavelength accuracy, the observed intensity of the fluorescent lamp light may vary. This may prevent you from accurately determining the peak wavelength.
- NOTICE 3:** If the energy level appears to be saturated during the determination of the wavelength accuracy, adjust the amount of light entering the monochromator by turning off some lamps in the laboratory.
- NOTICE 4:** In wavelength accuracy check, set the emission monochromator bandwidth at the standard (15 nm).

2. Check of wavelength accuracy of excitation monochromator
  - 2a. Attach the light sampling screw of the flow cell unit.
  - 2b. Set the fluorescence wavelength to the position determined in the procedure of 1l. above.
  - 2c. Turn on the lamp with the **PARAM SET** key.
  - 2d. Set PMT voltage “MED” with the **PARAM SET** key.
  - 2e. Make the Display Mode be Em < M > with the **UTILITY** key.
  - 2f. Set the wavelength of the excitation monochromator (Ex) at 380 nm with the **WAVE LENGTH** key.
  - 2g. Move the wavelength of the excitation monochromator (Ex) with the **WAVE LENGTH** key to obtain the wavelength where the displayed energy becomes its maximum.

**NOTICE:** Adjust the Xe lamp position properly, then check the wavelength accuracy of the excitation monochromator.

### Specification:

Obtained wavelength should be;

Ex: within  $\pm 3$  nm of 380 nm

Em: within  $\pm 3$  nm of 436 nm

## 4 Check of Sensitivity

### Content of Check:

Sensitivity of Fluorescence Detector is measured by measuring S/N value using Raman Spectrum of water.

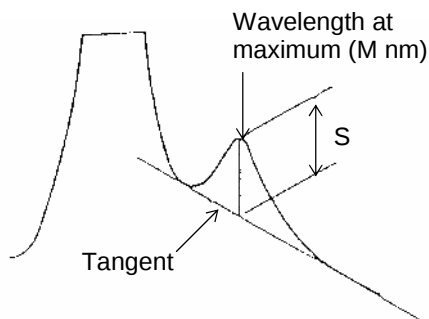
### Items to be Prepared:

1. Prepare the following.
  - 1a. A calibrated data processor for HPLC (Hitachi Model D-7500 Integrator or equivalent) or a recorder (Hitachi Model 056 Recorder or equivalent).
  - 1b. Calipers.
  - 1c. Load resistance coil (Teflon tube, inside diameter: 0.25 mm, length: 10 m).
2. Adjust the lamp position.
3. Flow ion exchanged water or distilled water with a flow rate of 1 mL/min for 3 minutes. Make the flow rate be 0 to stop the flow. Close the inlet and outlet of the Flow Cell tube. When flowing, connect a load resistance coil before the Flow Cell.
4. Connect the data processor for HPLC and Detector with the Signal Cable. When a recorder is used, connect the Signal Cable to Recorder terminals.

### Check Procedure:

1. Measurement of Raman spectrum of water
  - 1a. Turn on the Power switch and warm up the instrument for more than 1 hour.
  - 1b. Set the wavelength of the excitation monochromator (Ex) at 350 nm with the **WAVE LENGTH** key.
  - 1c. Set the recorder output speed at 60 nm/min with the **RECORD** key.
  - 1d. Set the recorder full scale range at 1000 with the **RECORD** key.
  - 1e. Set the time constant to 2.0 s, set the applied voltage to the photomultiplier to Medium with the **PARAM SET** key.
  - 1f. Set the scan mode to Em, set the fluorescence spectrum measurement wavelength at 350 nm to 450 nm with the **SPECT PARAM** key.
  - 1g. Start scanning, and input memory number to be used for storing with the **SPECT MEMO** key.
  - 1h. Set the spectrum number of output data, and background data with the **RECORD** key. Output the spectra with the **START STOP** key. Start the data processor or recorder at the same time for recording.

- 1i. Obtain Raman peak intensity (s) and wavelength at the maximum (M nm).



**NOTICE 1:** Use the Model D-7500 Integrator with the following conditions.

ATT: 5

Chart speed: 60 mm/min

Sampling period: 400 msec

**NOTICE 2:** When the Detector and Integrator are connected with the D-line (digital), start the output with the ALL START key of the Integrator.

2. Measurement of noise
- 2a. Keep the excitation wavelength at 350 nm, set the fluorescence wavelength at M nm with the WAVE LENGTH key.
- 2b. Start the Integrator/Recorder, record for 15 minutes.
- 2c. From the recorded chart, calculate the noise following ASTM E685-79 (1979).

**NOTICE:** Use the Model D-7500 Integrator with the following conditions:

ATT: 0

Chart speed: 10 mm/min

Sampling period: 400 msec

3. Calculation of S/N value (sensitivity) of Raman spectrum of water

$$S/N \text{ (value)} = \frac{S}{N}$$

**Specification:**

200 or more

**NOTICE:** Quality of distilled water and bubbles in water affect Raman spectra of water and noise level.  
 Select high quality water with no bubbles.  
 Incomplete adjustment of the optical system, a dirty optical system or Flow Cell may reduce S/N value.  
 If lamp replacement is not effective to increase S/N value, contact your nearest Service Office to ask adjustments.

### Remarks:

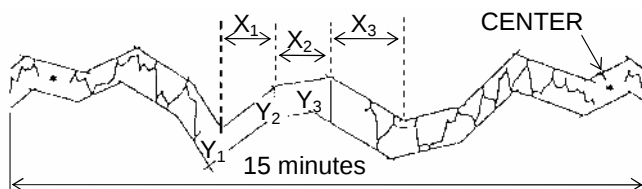
When the Model D-7500 Integrator is used, the voltage value corresponding to  $\dot{O}S\dot{O}$  can be calculated from the length of S (L mm) on the chart, and the noise level can be calculated from the noise width (mm) on the chart.

$$S = \frac{L}{152} \times 2^x (\text{mV})$$

$$N = \frac{\text{Noise width}}{152} \times 2^x (\text{mV})$$

Where; L is the length of S (mm)

X is the ATT (attenuator) setting on the Integrator,



$$\text{Noise level} = \sum_{R=1}^{R=n} Y_R / n (\text{Unit of } X_n \text{ is 1 min})$$

Where:  $Y_R$  is a length (mm) on chart at each time period.  
 (Extracted from ASTM E685-79 (1979))

