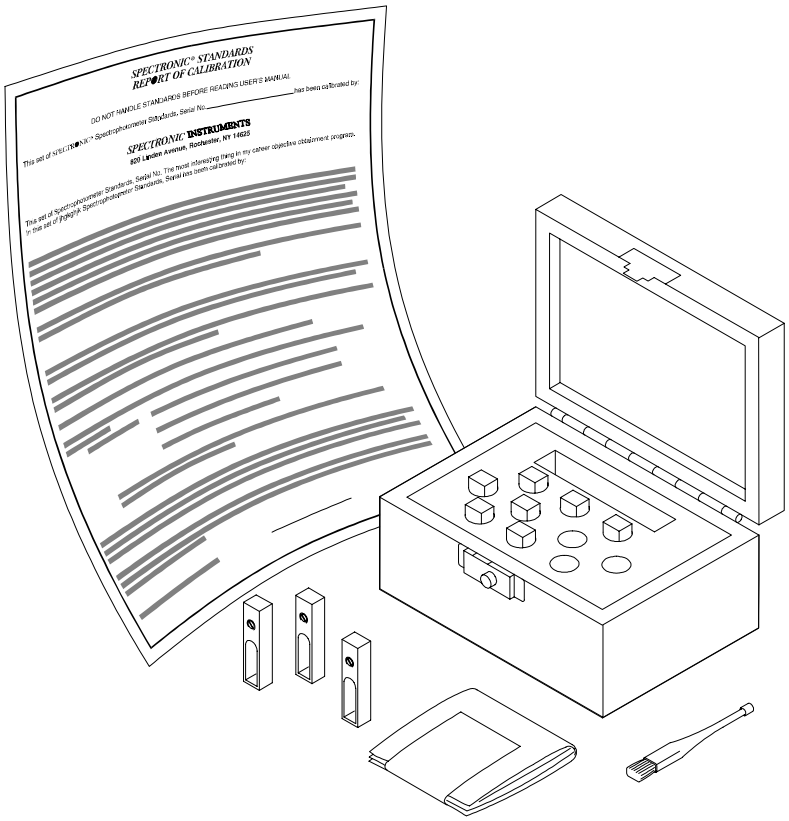
SPECTRONIC® STANDARDS



Thermo Spectronic

OPERATOR'S MANUAL

Table of Contents

INTRODUCTION

SPECTRONIC Standards are an affordable, accurate and stable set of filters for performance validation purposes.

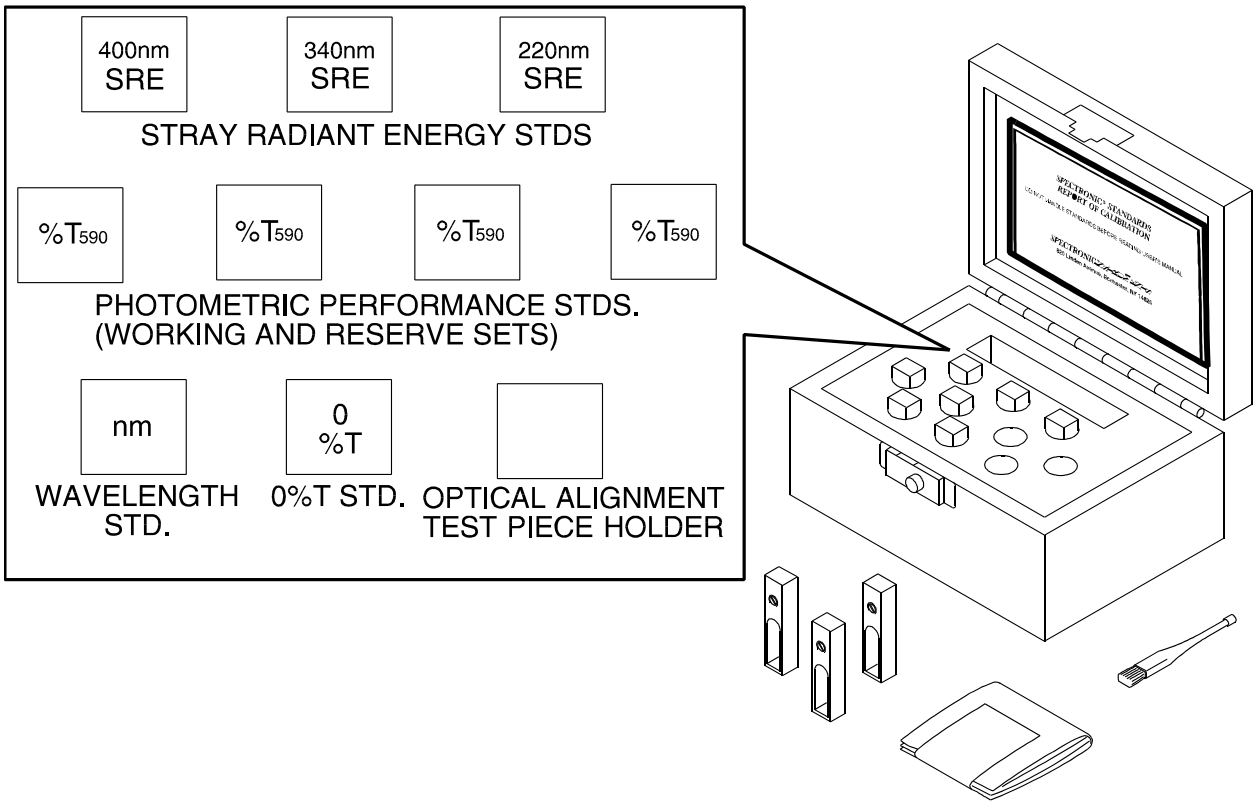
SPECTRONIC Standards enable the spectrophotometer user to quickly and reliably evaluate the major performance parameters of SPECTRONIC spectrophotometers. As a part of the laboratory's normal quality control program, SPECTRONIC Standards are an invaluable aid in the detection of incipient instrument problems before errors occur.

DESCRIPTION

SPECTRONIC Standards set 333150 consists of the following items (see Figure 1):

- 220nm stray radiant energy standard (labeled 220nm SRE)
- 340nm stray radiant energy standard (labeled 340nm SRE)
- 400nm stray radiant energy standard (labeled 400nm SRE)
- Wavelength standard (individually labeled XXX.Xnm)
- 0% transmittance standard (labeled 0% T)
- Two photometric performance standards with stated transmittance values near 10% T at 590nm (individually labeled XX.X%T_{590})
- Two photometric performance standards with stated transmittance values near 50% T at 590nm (individually labeled XX.X%T_{590})
- Optical alignment test piece (unlabeled)
- Cleaning brush
- Pack of photosensitive paper 333152
- Carrying and storage case
- Certificate of calibration

Figure 1, SPECTRONIC Standards Set



All standards have the same external dimensions as a 10mm-pathlength square cuvette, so they will fit in practically any spectrophotometer that accepts such cuvettes. Adapters that permit the standards to be used in Thermo Spectronic test-tube-holder sample compartments are described under Accessories.

Your set of SPECTRONIC Standards has been individually tested and certified. Wherever pertinent, SPECTRONIC Standard values are traceable to N.I.S.T.

0% Transmittance Standard

The 0% transmittance (0%T) standard is opaque at all spectrophotometric wavelengths. It is used to check the 0%T reading by blocking the light beam from the instrument's source lamp. This constitutes a test for light leaks and for incorrect adjustment of the zero setting. The 0% transmittance standard's apparent transmittance, if any, is also used to refine the estimate of stray radiant energy in instruments that do not read exactly 0.0%T.

This standard is not traceable to N.I.S.T.

Some spectrophotometers do not provide the user with a transmittance mode of operation. If only an absorbance mode is available, readings equivalent to nearly 0%T will be out of range. Therefore, the 0% transmittance standard cannot be used in such instruments.

Wavelength Standard

The wavelength standard produces three widely spaced, symmetrical, high transmittance peaks in the regions of 400nm, 525nm and 780nm. The primary testing peak, near 525nm, is located in the region where many spectrophotometers show the flattest energy measurement response with small changes in wavelength. The primary peak is recorded on the label of the wavelength standard and is the certified wavelength for your wavelength standard recorded on the certificate accompanying the SPECTRONIC Standards. This primary peak near 525nm is the only peak traceable to the Thermo Spectronic calibration spectrophotometer which has been standardized to be within ±0.1nm on the 486.0nm and 656.1nm deuterium lines and the 253.7nm and 546.1nm mercury lines.

The peaks in the regions of 400nm and 780nm can be used for checking the repeatability of your instrument over a wider wavelength range.

Stray Radiant Energy Standards

Three stray radiant energy standards are provided. Each standard is essentially opaque at its test wavelength and highly transmitting at longer wavelengths, as shown in Figure 2.

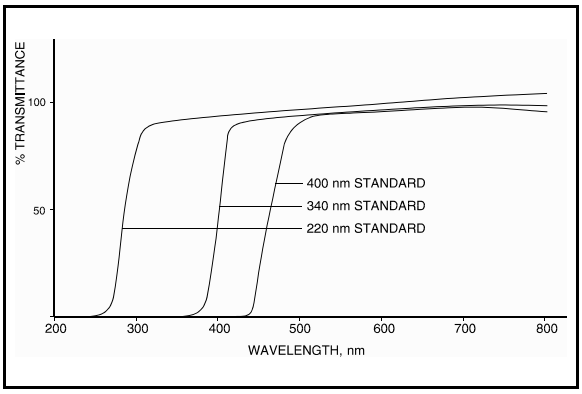


Figure 2. Transmittance/Wavelength Profile of Stray Radiant Energy Standards

The test wavelengths chosen (220nm, 340nm and 400nm) correspond to points of low energy for tungsten and deuterium lamps, so tests measure worst-case stray radiant energy levels for both types of lamp. Stray radiant energy standards are not traceable to N.I.S.T.

Photometric Performance Standards

The photometric performance standards test photometric accuracy, linearity, and precision. Transmittance of these standards is extremely constant with changes in wavelength in the region of 590nm, as shown in Figure 3. Consequently, no bandwidth compensation is required, and photometric errors can be distinguished from wavelength errors.

Four individually certified standards are supplied in each set: two with transmittance values of approximately 50%T and two with values of approximately 10%T.

Because any dirt or scratches on the neutral glass can affect the transmittance value of a standard, the 50%T and 10%T standards with white labels (the working pair) should be used for all instrument tests. The other pair (with yellow labels) should be kept in reserve and used only to periodically verify the accuracy of the working pair. These values are traceable to N.I.S.T. SRM 930d and SRM 1930.

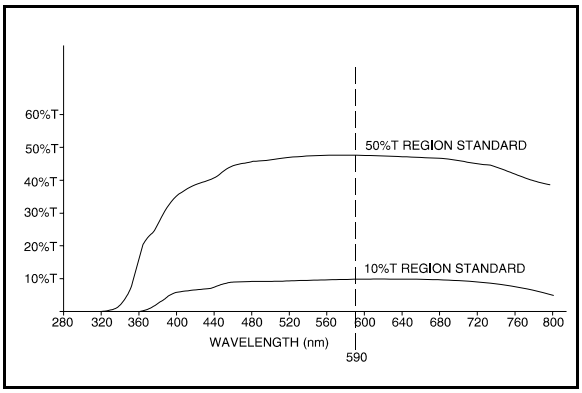


Figure 3. Transmittance/Wavelength Profile of Photometric Performance Standards

Optical Alignment Test Piece

The optical alignment test piece is an empty filter holder physically identical to the holders for the other standards. Consequently, it can be used when setting 100%T to make sure that the filter holders of the other standards do not affect transmittance values.

It can also be loaded with the photosensitive paper supplied to obtain a permanent record of beam location and thus check for agreement between the location of the cuvette window and that of the optical beam.

Recertification and Repair Service

Thermo Spectronic offers a recertification and repair service for the SPECTRONIC Standards. It is recommended that you return your standards for recertification every two years — or whenever you have reason to believe that a standard no longer exhibits its certified value. Before returning your standards, contact your local distributor.

HANDLING AND CARE OF STANDARDS

The transmittance of the photometric performance ($XX.X\%T_{590}$) standards is significantly affected by the condition of the optical surfaces. Any dirt (lint, fingerprints, oil, dust, smoke film, etc.) can cause erroneous test results. Handling the photometric performance standards to clean them involves a risk of damaging the surfaces. So, prevention of soiling is far better than cleaning.

Always keep the standards in their case when not in use. Always keep the cover closed. Never expose the standards to airborne smoke, dust, oil vapor or chemical fumes.

Before using the photometric performance standards, visually inspect them. Remove any dust or lint by gentle brushing with the cleaning brush supplied. To keep the brush itself clean, do not handle its bristles, and do not use it for any purpose other than dusting the standards.

If it becomes necessary to clean a photometric performance standard with a liquid, clean only one standard at a time, and proceed as follows:

1. Read and record the standard value in percent transmittance (%T) at 590nm before cleaning.
2. Use a nut driver to loosen the nut securing the glass filter. Back the nut out almost all the way, but do not remove it.
3. Remove the filter, holding it by the edges and being careful not to scrape the glass against the filter holder — especially the metal lip that projects near the bottom of the holder.

NOTE

You must use the appropriate liquid when cleaning the glass:

- Photometric performance standards - use only **reagent quality isopropyl alcohol**
- Wavelength or stray radiant energy standards - use only **distilled or deionized water**

4. Clean the glass using only the recommended liquid and a soft, clean, lint-free cloth.
5. Reassemble the filter and inspect it for cleanliness and scratches.
6. Read the standard value at 590nm. The percent transmittance (%T) reading should remain the same or increase. If the value has decreased, repeat the cleaning process.

OPERATING TECHNIQUE

The test results you obtain using SPECTRONIC Standards will be influenced by your technique in operating the instrument as well as by the condition of the instrument. Refer to the instrument operating manual to make sure you use the instrument correctly and thus avoid introducing errors into the test results.

INSTRUMENT SETUP

Set up the spectrophotometer as indicated in the operator's manual to make transmittance measurements, being sure to allow the recommended warm-up time.

Make all settings and readings as accurately as possible. This will minimize the borderline normal/abnormal results which give no clear indication of whether the spectrophotometer needs professional service or not.

The SPECTRONIC Standards have the same outside dimensions as a 10mm square cuvette. If necessary, install an appropriate adapter (see below) in the instrument's sample compartment.

Adapter and Accessory Filters for SPECTRONIC 20 Series Spectrophotometer

To use the SPECTRONIC Standards with the SPECTRONIC 20 or 20$^+$ series spectrophotometers, the 333178 Standard Adapter (for ½-inch square cuvettes) must first be installed. Cuvette holder 333176 is also required. (The 333178 Adapter is standard on all current models of the SPECTRONIC 20. Pre-1990 models will not accept the 333176 ½-inch Cuvette Holder, so it should be replaced with the latest 333178 Adapter.)

Insert standards into the cuvette holder as shown in Figure 4. Orient the holder so that the window side of the standard faces to the right. Push the holder down into the sample compartment as far as possible, and close the sample compartment lid.

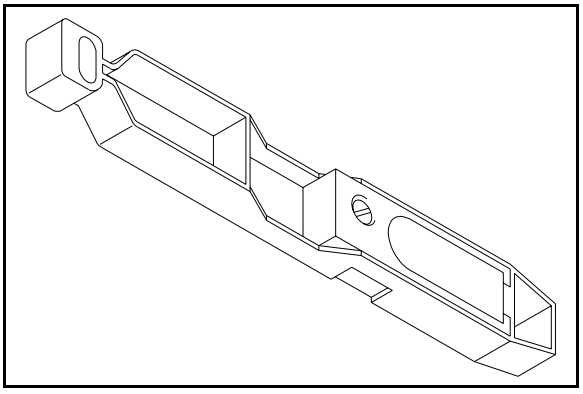


Figure 4. Standard Inserted in Cuvette Holder 333176 for SPECTRONIC 20 or 20$^+$ Series Spectrophotometers

It is highly recommended that the Accessory Filter Kit 333129 (for all SPECTRONIC 20 or 20$^+$ series) be used to optimize the performance of the spectrophotometers — both in routine use and during tests with SPECTRONIC Standards.

Adapter for SPECTRONIC 21 Spectrophotometer

If your instrument is equipped with a General Purpose Sample Compartment (a module attached to the left of the main instrument chassis), the SPECTRONIC Standards can be used without an adapter.

To use the SPECTRONIC Standards in the Universal Test Tube Holder built into the main instrument, Cuvette Adapter 332208 is required (supplied with instrument). Insert standards into the adapter as shown in Figure 5. Be sure to press the standard flush against the inside wall of the cuvette adapter.

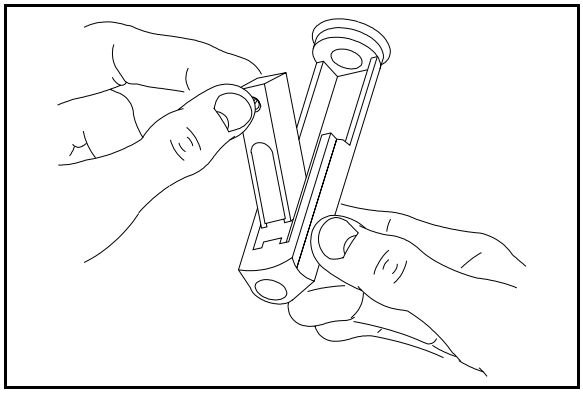


Figure 5. Inserting a Standard into Cuvette Adapter 332208 for SPECTRONIC 21 Spectrophotometers

To insert the adapter into the sample compartment, orient the fiducial mark on the adapter so that it points to the right and is parallel with the front edge of the instrument. Push the adapter straight down into the compartment without turning the adapter at all. It will self-align. Close the sample compartment lid.

RECORDING AND INTERPRETING TEST RESULTS

Recording Test Results

Test results should be recorded by date in a test log — one log for each instrument to be inspected. (A sample of such a log is provided at the end of this manual.) By scanning the log entries, the user can detect gradual changes of test values over a period of time. Any conditions or events that might affect test results (for example, cleaning or lamp replacement) should be recorded in the Comments column.

Interpreting Test Results

Although every effort is made to manufacture and measure the standards as accurately as possible, the wavelength and photometric performance standards have tolerances associated with them.

In interpreting test results to evaluate instrument performance, the instrument tolerance and the standard tolerance should be added to determine the acceptable total deviation from nominal. For convenience, Table 1 gives the maximum deviations from nominal (instrument tolerance plus standard tolerance plus allowance for indeterminate error in the test procedure) for each SPECTRONIC Standard used with each SPECTRONIC spectrophotometer.

When using these standards in spectrophotometers not manufactured by Thermo Spectronic, a table similar to Table 1 should be constructed for each test piece by adding the instrument specification to the tolerance stated in the certificate.

Table 1 Maximum Acceptable Deviations from Nominal for SPECTRONIC Standards used with SPECTRONIC Spectrophotometers *(Figures are the sum of the spectrophotometer tolerance, the standard tolerance and an [estimated] allowance for indeterminate variables.)*

	Test Standard				
SPECTRONIC Spectrophotometer	**Stray Radiant Energy**	**Wavelength Accuracy**	**0%T**	**Photometric Accuracy**	**Optical Alignment**
MINI 20	N/A	±5nm	0.0	N/A	*
20/20D/20^{+}/20D^{+} Series	0-0.85% T**	±4.5nm	0.0††	±3.0% T	*
21 MV/DV/DUV	0-0.4% T	±5nm	0.0†	±3.0% T	*
301/401	0-0.45% T	±4nm	0.0†	±1.2% T	*
501/601	0-0.4% T	±3nm	0.0†	±1.2% T	*
1001/1001+/1201	0-0.4% T	±3nm	0.0†	±1.2% T	*
GENESYS 2 or 5	0-0.4% T	±3nm	0.0†	±1.2% T	*
GENESYS 20	0-0.45% T	±4nm	0.0†	±1.2% T	*
GENESYS 10 Vis	0-0.45% T	±4nm	0.0†	±1.2% T	*
GENESYS 10 UV	≤0.1% T	±3nm	≤0.1% T	±1.2% T	*
GENESYS 10 UV*scanning*	≤0.1% T	±3nm	≤0.1% T	±1.2% T	*
GENESYS 6	≤0.1% T	±3nm	≤0.1% T	±1.2% T	*
BioMate 3	≤0.1% T	±3nm	≤0.1% T	±1.2% T	*

* Results are pictorial. All of the energy should strike the optical surface of the cell. The size and shape of the beam should be constant for the life of the instrument.
** With Accessory Filter Kit 333128 or 333129 — highly recommended accessories
† An offset of ±0.1%T is acceptable.
†† An offset of ±0.2%T is acceptable.

Use of Absorbance Mode

The instructions in this manual assume that the spectrophotometer is used in the transmittance mode. Test results can be read in absorbance and converted mathematically to transmittance*, but the following limitations must be considered:

1. Readings near 0%T will be overrange in absorbance. Therefore, the 0%T standard cannot be used.
2. Estimates of stray radiant energy will be less accurate because they normally will be overrange in absorbance.

Because of these limitations, it is recommended that the transmittance mode be used for any spectrophotometer on which it is available.

The accuracy of wavelength tests is the same in either the transmittance or absorbance mode. A maximum in transmittance is a minimum in absorbance, so the wavelength peaks will be seen as minima in the absorbance mode. The wavelength of a peak is the same in either mode.

* Equations for interconversion of absorbance readings and transmittance readings:

$$T = \frac{1}{10^{A}} = 10^{-A} \qquad (1)$$

where T = %T/100

$$A = -\log_{10} T \qquad (2)$$

where A = absorbance
T = transmittance
%T = percent transmittance

TEST PROCEDURES

0% Transmittance and Stray Radiant Energy Test

SPECTRONIC 20/20^{+} Series Spectrophotometers

1. On SPECTRONIC 20 and 20D models only, ensure that the correct phototube is installed.
2. Set the wavelength to 400nm.
3. On SPECTRONIC 20^{+} and 20D^{+} models only, set the filter lever to the correct wavelength range.
4. Ensure that the sample compartment is empty.
5. On SPECTRONIC 20, 20D and 20^{+} models only, use the OFF-ON/0%T knob to set the readout to 0%T.
6. Insert the optical alignment test piece into the 333176 holder and then place in the sample compartment.
7. Use the front right knob or 100%T/0A knob to set the readout to 100%T.
8. Replace the optical alignment test piece with the 0%T standard. Record the 0%T reading.
   If the 0%T reading exceeds the Maximum Acceptable Deviations as listed in Table 1, correct it and repeat steps 1 through 8 before proceeding.
9. Replace the 0%T standard with the SRE standard whose wavelength corresponds to the setting in step 2. Observe the %T reading.
10. Subtract the reading in step 8 from the reading in step 9. Record the result, which is the stray radiant energy at the test wavelength. This value should conform to the summed tolerances given in Table 1.
11. Repeat the procedure using a wavelength of 340nm in step 2.

Other spectrophotometer models

1. Set the wavelength to 400nm.
2. Set 100%T.
3. Insert the 0%T standard and record the 0%T reading.
   If the 0%T reading exceeds the Maximum Acceptable Deviations as listed in Table 1, correct it and repeat steps 1 through 3 before proceeding.
4. Replace the 0%T standard with the SRE standard whose wavelength corresponds to the setting in step 1. Observe the %T reading.
5. Subtract the reading in step 3 from the reading in 4. Record the result, which is the stray radiant energy at the test wavelength. This value should conform to the summed tolerances given in Table 1.
6. Repeat the procedure using the wavelengths specified for your instrument in Table 2.

Table 2 Wavelengths to use when testing 0% transmittance and stray radiant energy

SPECTRONIC Spectrophotometer	Wavelengths
MINI 20	400nm
SPECTRONIC 20/20D/20$^+$/20D$^+$	400nm, 340nm
SPECTRONIC 21 MV	400nm, 340nm
SPECTRONIC 21 DUV	400nm, 340nm, 220nm
SPECTRONIC 301/401	400nm, 340nm
SPECTRONIC 501	400nm, 340nm
SPECTRONIC 601	400nm, 340nm, 220nm
SPECTRONIC 1001 series/1201	400nm, 340nm, 220nm
GENESYS 2 or 5	400nm, 340nm, 220nm
GENESYS 20	400nm, 340nm
GENESYS 10 Vis	400nm, 340nm
GENESYS 10 UV	400nm, 340nm, 220nm
GENESYS 10 UV*scanning*	400nm, 340nm, 220nm
GENESYS 6	400nm, 340nm, 220nm
BioMate 3	400nm, 340nm, 220nm

Wavelength Accuracy Test

Note: With the SPECTRONIC 20/20D spectrophotometers, the phototube and second-order filter must be manually installed by the user. For these instruments, follow the instructions given under *Wavelength Peak Selection for SPECTRONIC 20/20D Spectrophotometers* when performing steps 2 and 5 of the General Procedure.

General Procedure

1. If the instrument has more than one source lamp, turn on the tungsten lamp. Insert the wavelength standard in the instrument.
2. Refer to the certificate provided with your set of SPECTRONIC Standards. Set the wavelength display to read approximately 10nm lower than the certified wavelength of the Primary Wavelength Test Peak indicated on the certificate.
3. Adjust the 100%T control to give a reading that is on scale, preferably in the 40 to 50% region. When using a digital readout spectrophotometer, use the absorbance mode to set .3A to .4A. Then switch back to % transmittance.
4. While watching the %T display, slowly change the wavelength toward the certified wavelength. The %T reading should increase. If not, go back to step 2 and use a lower wavelength.
   Find the wavelength at which the transmittance is highest and record the wavelength (not the transmittance) where this peak is found. This wavelength should match the certified wavelength within the summed tolerances given in Table 1.
   The 100%T control may be readjusted as needed to keep the peak reading on scale because the transmittance value is unimportant to the test. For consistency, the peak should be approached each time from the same direction; that is, from a wavelength below that of the peak. Practice this a few times to improve technique.
5. Repeat steps 2 and 4 for each of the other two solid-line peaks on the certificate. These two peaks are not certified, but can be used to check repeatability. The wavelengths may not be within the summed tolerances given in Table 1. They are approximations only. Record the wavelengths as seen on the spectrophotometer and use to check repeatability only.

Wavelength Peak Selection for SPECTRONIC 20/20D Spectrophotometers

- With visible phototube 332971, use the first-order peaks near 525 and 400nm. This phototube is "blind" in the 780nm region.
- With wide-range phototube 332989, use only the peaks near 525 and 400nm.
- With infrared phototube 332972, use only the peaks near 525 and 780nm.

Note: By removing the second-order-blocking filter (part of Accessory Filter Kits 333128 and 333129), the second order of the peak in the 400nm region can be found near 800nm and used as the third point.

Wavelength Peak Selection for SPECTRONIC 20$^+$/20D$^+$ Spectrophotometers

- Ensure that the filter lever is positioned to the correct wavelength range.

NOTE

For greater accuracy on non-scanning instruments, a point-by-point scan can be used. Read and plot the filter transmittance at a series of several discrete, closely-spaced wavelength settings around the expected location of a transmission peak. Carefully reset 100%T at each wavelength with the filter removed from the light beam. The advantage of this procedure is that any slope in the instrument's spectral response curve will have less effect on where the peak is found than it would in the method of direct, continuous scanning without resetting 100%T. The disadvantage is that it is time-consuming and tedious; many settings and readings must be carefully made and plotted to obtain one result.
Our recommendation is to use the method given in the *General Procedure* rather than try to minimize error through this more time-consuming method.

Photometric Accuracy/Linearity Test

1. Set the wavelength to 590nm.
2. On SPECTRONIC 20, 20D and 20$^+$ models only, set 0%T with the sample compartment empty.
3. Insert the optical alignment test piece into the 333176 holder and then place into the sample compartment.
4. Adjust the instrument to read exactly 100%T.
5. Remove the test piece. On the SPECTRONIC 20D$^+$ model only, the 100%T reading should remain essentially unchanged.
   SPECTRONIC 20, 20D and 20$^+$ spectrophotometers only: Remove the standard from the holder and reinsert the empty holder to open the built-in occluder. When the empty holder is inserted, the 100%T reading should remain essentially unchanged.
6. Replace the optical alignment test piece with the photometric performance standard whose labeled value is near 50%T. Record the instrument reading. The reading should be within the summed tolerance given in Table 1.
7. Replace the 50%T standard with the standard whose labeled value is near 10%T. Record the instrument reading. The reading should be within the summed tolerance given in Table 1.

Optical Alignment Test

1. Turn the spectrophotometer off.
2. In subdued light, open the pack of light-sensitive paper (Cat. No. 335152) supplied with the SPECTRONIC Standards.
3. Remove a sheet of paper and cut it to fit behind the window in the test piece. (A piece of index card stock of the same size can be used as rigid backing for the paper.)
4. Loosen the screw holding the filter clip in the test piece.
5. Slide the light-sensitive paper under the clip with the yellow side of the paper facing out through the window.
6. Tighten the screw.
7. Insert the optical alignment test piece in the sample compartment.
8. Set the wavelength to 425nm.

9. *On instruments that use an occluder*, including SPECTRONIC 20, 20$^+$, and 20D spectrophotometers, turn the 100%T control to the maximum clockwise position. *On all other SPECTRONIC spectrophotometers*, proceed with step 10.
10. Turn the instrument on and expose the paper to the light beam from the instrument for 3 to 24 hours. (The optimum exposure time depends on the light level of the particular instrument, but is not critical.)

Note: The yellow side of the paper must be toward the light source.

11. Remove the standard. The exposed area of the paper should be white. The unexposed area should remain yellow.
12. To develop the paper, expose it (while mounted in the standard) to ammonia fumes until the unexposed area turns dark blue.
13. Visually compare the beam image with one of your cuvettes or sample cells. The beam should be entirely within the optical window.
14. To record the position of the beam image relative to the window in the standard, draw a line on the paper around the inside edge of the window. Remove the paper from the standard.
15. Date, label and save the paper for comparison with future tests. It is convenient to tape the dated papers in sequence on the instrument's test log.

ACCESSORIES

For SPECTRONIC 20 and 20$^+$ series spectrophotometers

NOTE: Both 333178 and 333176 are necessary to accommodate SPECTRONIC Standards.

333178 ½-inch Sample Compartment Adapter
333176 Square Cuvette Holder
333129 Accessory Filter Kit for SPECTRONIC 20 and 20$^+$ series (recommended to reduce stray radiant energy)

For SPECTRONIC 21 spectrophotometer without a General Purpose Sample Compartment

332208 Cuvette adapter