

**USEPA<sup>1</sup> Bicinchoninate Method<sup>2</sup>****0.04 to 5.00 mg/L Cu****Method 8506 (CuVer 1) and Method 8026 (CuVer 2)****Powder Pillows or AccuVac<sup>®</sup> Ampuls**

**Scope and application:** For water, wastewater and seawater<sup>3</sup>; Method 8506 USEPA approved for reporting wastewater analysis (digestion required)<sup>4</sup>

<sup>1</sup> Approved, USEPA and Standard Method 3500 Cu C or E.

<sup>2</sup> Adapted from Nakano, S., Yakugaku Zasshi, 82 486-491 (1962) [Chemical Abstracts, 58 3390e (1963)].

<sup>3</sup> Pretreatment required for the powder pillow method- refer to the Interference section.

<sup>4</sup> Federal Register, 45 (105) 36166 (May 29, 1980).

**Test preparation****Before starting**

To make sure that all forms of the metal are measured, digest the sample with heat and acid. Use the mild or vigorous digestion. Refer to the Water Analysis Guide for more information.

Always do tests in sample cells or AccuVac<sup>®</sup> Ampuls. Do not put the instrument in the sample or pour the sample into the cell holder.

Make sure that the sample cells are clean and there are no scratches where the light passes through them.

Rinse the sample cell and cap with the sample three times before the sample cell is filled.

Make sure that there are no fingerprints or liquid on the external surface of the sample cells or AccuVac<sup>®</sup> Ampuls. Wipe with a lint-free cloth before measurement.

Cold waters can cause condensation on the sample cell or bubbles in the sample cell during color development. Examine the sample cell for condensation or bubbles. Remove condensation with a lint-free cloth. Invert the sample cell to remove bubbles.

Install the instrument cap over the cell holder before ZERO or READ is pushed.

After the test, immediately empty and rinse the sample cell. Rinse the sample cell and cap three times with deionized water.

For the best results, measure the reagent blank value for each new lot of reagent. Replace the sample with deionized water in the test procedure to determine the reagent blank value. Subtract the reagent blank value from the sample results.

The AccuVac Ampul Snapper makes AccuVac Ampul tests easier to do. The AccuVac Ampul Snapper keeps the broken tip of the ampul, prevents exposure to the sample and provides controlled conditions for filling the ampule.

Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.

Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

**Items to collect****Powder pillows**

Description	Quantity
CuVer <sup>®</sup> 1 Copper Reagent Powder Pillow, 10-mL	1
Sample cells, 25-mm (10 mL)	2

Refer to Consumables and replacements items on page 6 for order information.

## AccuVac Ampuls

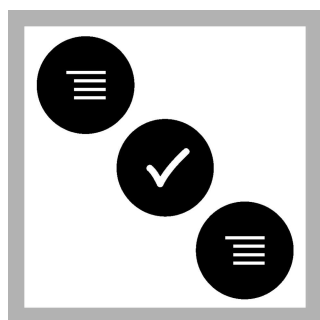
Description	Quantity
CuVer® 2 Reagent AccuVac® Ampul	1
Beaker, 50-mL	1
Stopper for 18-mm tubes and AccuVac Ampuls	1
Sample cell, 25-mm (10 mL)	1

Refer to Consumables and replacements items on page 6 for order information.

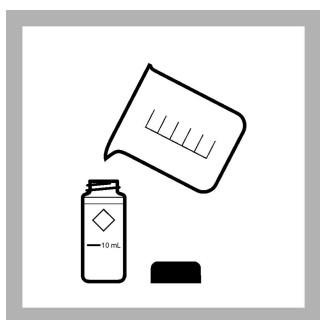
## Sample collection and storage

- Collect samples in clean glass or plastic bottles that have been cleaned with 6 N (1:1) hydrochloric acid and rinsed with deionized water.
- To preserve samples for later analysis, adjust the sample pH to less than 2 with concentrated nitric acid (about 2 mL per liter). No acid addition is necessary if the sample is tested immediately.
- If only dissolved copper is to be determined, filter the sample before the acid addition.
- Keep the preserved samples at room temperature for a maximum of 6 months.
- Before analysis, adjust the pH to 4–6 with 8.0 N potassium hydroxide standard solution (do not exceed pH 6, as copper may precipitate).
- Correct the test result for the dilution caused by the volume additions.

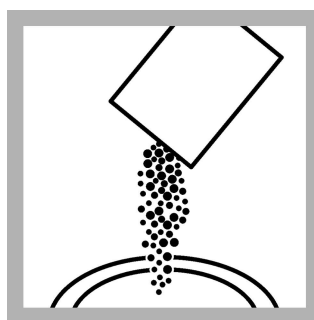
## Powder pillow procedure (Method 8506)



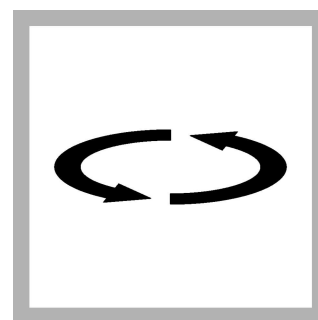
1. Set the instrument to channel 1. Refer to the instrument documentation.



2. **Prepare the sample:** Fill a sample cell to the 10-mL mark with sample.



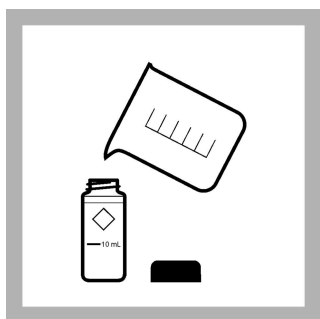
3. Add one CuVer 1 Copper Reagent powder pillow to the sample cell.



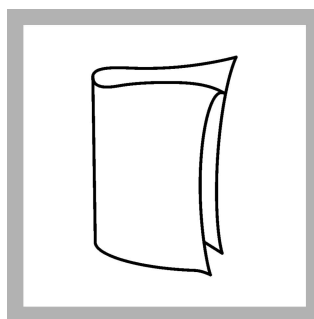
4. Swirl to mix.



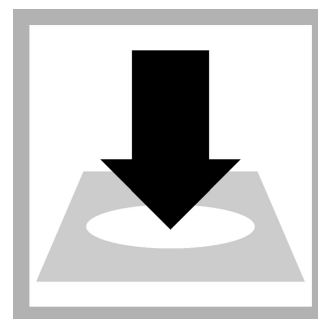
5. Set and start a timer for 2 minutes. A 2-minute reaction time starts.



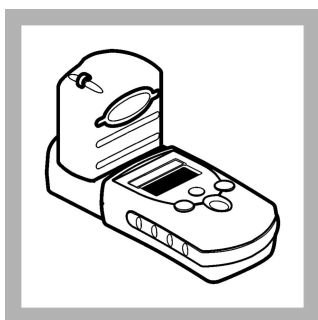
6. **Prepare the blank:** Fill a sample cell to the 10-mL mark with sample. Close the sample cell.



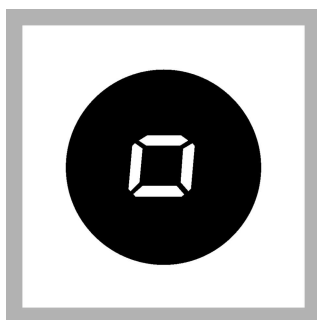
7. When the timer expires, clean the blank sample cell.



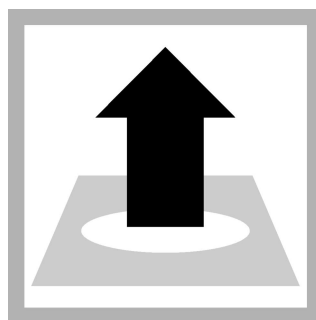
8. Insert the blank into the cell holder. Point the diamond mark on the sample cell toward the keypad.



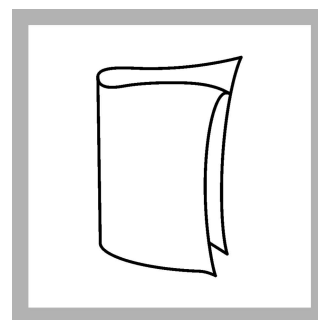
9. Install the instrument cap over the cell holder.



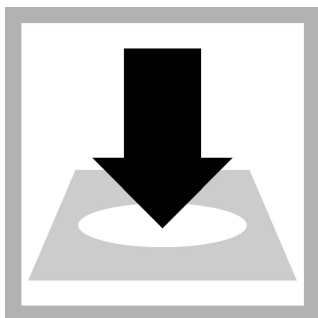
10. Push **ZERO**. The display shows "0.00".



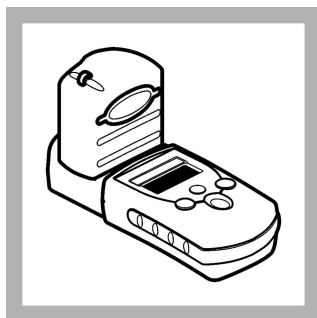
11. Remove the sample cell from the cell holder.



12. Clean the prepared sample cell.



13. Insert the prepared sample into the cell holder. Point the diamond mark on the sample cell toward the keypad.

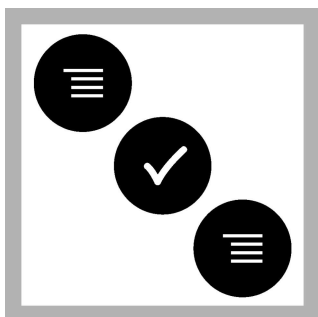


14. Install the instrument cap over the cell holder.

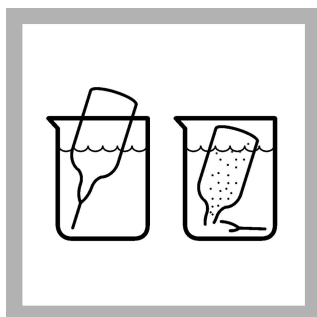


15. Push **READ**. Results show in mg/L copper (Cu).

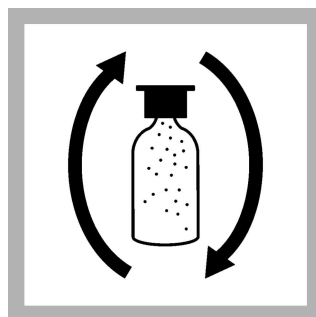
### AccuVac<sup>®</sup> Ampul procedure (Method 8026)



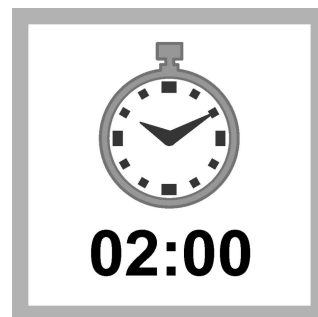
1. Set the instrument to channel 2. Refer to the instrument documentation.



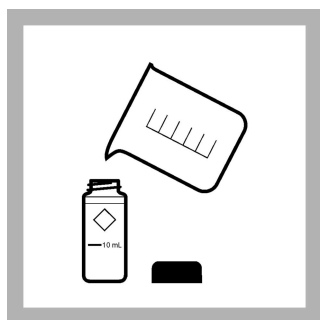
2. **Prepare the sample:** Collect at least 40 mL of sample in a 50-mL beaker. Fill the AccuVac Ampul with sample. Keep the tip immersed while the AccuVac Ampul fills completely.



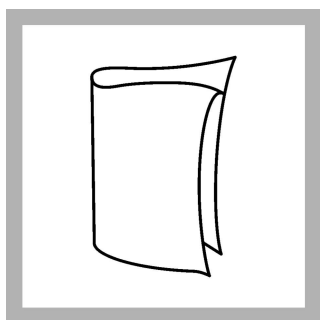
3. Quickly invert the AccuVac Ampul several times to mix.



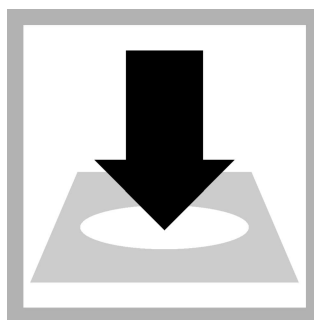
4. Set and start a timer for 2 minutes. A 2-minute reaction time starts.



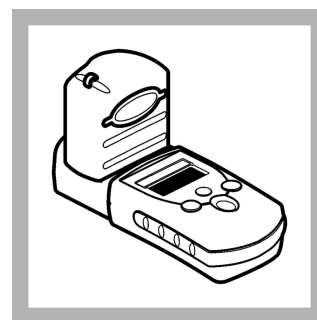
**5. Prepare the blank:** Fill a sample cell to the 10-mL mark with sample. Close the sample cell.



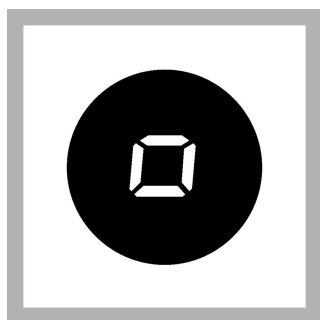
**6.** When the timer expires, clean the blank sample cell.



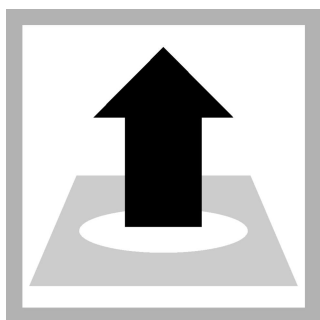
**7.** Insert the blank into the cell holder. Point the diamond mark on the sample cell toward the keypad.



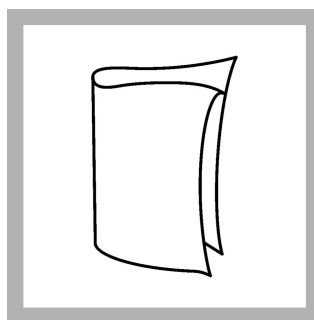
**8.** Install the instrument cap over the cell holder.



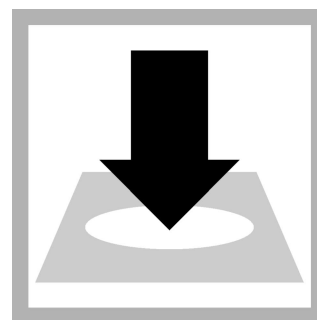
**9.** Push **ZERO**. The display shows "0.00".



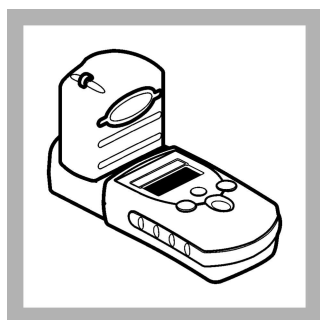
**10.** Remove the sample cell from the cell holder.



**11.** Clean the AccuVac Ampul.



**12.** Insert the prepared sample AccuVac Ampul into the cell holder.



**13.** Install the instrument cap over the cell holder.



**14.** Push **READ**. Results show in mg/L copper (Cu).

## Interferences

Table 1 gives treatments for powder pillows. Table 2 gives treatments for AccuVac Ampuls. To differentiate free copper from that complexed to EDTA or other complexing agents, add Free Copper Reagent Powder Pillow to 25 mL of sample in a 25-mL mixing cylinder. Transfer the solution to a 10-mL sample cell for measurements. The result will be free copper only.

To determine total dissolved copper (free and complexed), return the 10-mL sample cell portion to the mixing cylinder and add a Hydrosulfite Reagent Powder Pillow. Stopper and invert to mix. Once again, transfer solution to a 10-mL sample cell for measurements. The result will be total dissolved copper (free and complexed). Unlike CuVer 1 Reagent,

CuVer 2 Reagent Powder Pillows and AccuVac Ampuls react directly with copper that is complexed by chelants such as EDTA.

**Table 1 Interfering substances and suggested treatments for powder pillows**

Interfering substance	Interference level
Acidity	If the sample is extremely acidic (pH 2 or less), a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution by drops until the sample pH is between 4 and 6, then start the test.
Aluminum, Al <sup>3+</sup>	Use the powder pillow procedure, but add a CuVer 2 Copper Reagent Powder Pillow to 25 mL of sample in a 25-mL mixing cylinder and not the CuVer 1 Pillow. After color development, transfer this solution to a 10-mL sample cell for copper measurements. Results obtained will include total dissolved copper (free and complexed).
Cyanide, CN <sup>-</sup>	Prevents full color development. Before the CuVer 1 Powder Pillow Reagent is added, add 0.2 mL of formaldehyde to the 10-mL sample. Wait 4 minutes, then take the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Use the powder pillow procedure, but add a CuVer 2 Copper Reagent Powder Pillow to 25 mL of sample in a 25-mL mixing cylinder and not the CuVer 1 Pillow. After color development, transfer this solution to a 10-mL sample cell for copper measurements. Results obtained will include total dissolved copper (free and complexed).
Iron, Fe <sup>3+</sup>	Use the powder pillow procedure, but add a CuVer 2 Copper Reagent Powder Pillow to 25 mL of sample in a 25-mL mixing cylinder and not the CuVer 1 Pillow. After color development, transfer this solution to a 10-mL sample cell for copper measurements. Results obtained will include total dissolved copper (free and complexed).
Silver, Ag <sup>+</sup>	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution to 75 mL of sample, then filter through a fine or highly retentive filter. Use the filtered sample in the procedure.

**Table 2 Interfering substances and suggested treatments for AccuVac Ampuls**

Interfering substance	Interference level
Acidity	If the sample is extremely acidic (pH 2 or less), a precipitate may form. Add 8 N Potassium Hydroxide Standard Solution by drops until the sample pH is between 4 and 6, then start the test.
Aluminum, Al <sup>3+</sup>	Reagents accommodate high levels.
Cyanide, CN <sup>-</sup>	Prevents full color development. Add 0.8 mL of formaldehyde per 40-mL of sample, then use the CuVer 2 Reagent AccuVac Ampul. Wait 4 minutes, then take the reading. Multiply the test results by 1.02 to correct for sample dilution by the formaldehyde.
Hardness	Reagents accommodate high levels.
Iron, Fe <sup>3+</sup>	Reagents accommodate high levels.
Silver, Ag <sup>+</sup>	If a turbidity remains and turns black, silver interference is likely. Add 10 drops of saturated Potassium Chloride Solution to 75 mL of sample, then filter through a fine or highly retentive filter. Use the filtered sample in the procedure.

## Accuracy check

### Standard additions method

Use the standard additions method to validate the test procedure, reagents and instrument and to find if there is an interference in the sample.

Items to collect:

- Copper Standard Solution Voluette Ampule, 75 mg/L
- Ampule breaker
- Pipet, TenSette®, 0.1–1.0 mL and tips
- Mixing cylinders, 25-mL (3)

1. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the standard solution, respectively, to three 25-mL portions of fresh sample. Mix well.  
**Note:** For AccuVac® Ampuls, add 0.2 mL, 0.4 mL and 0.6 mL of the standard solution to three 50-mL portions of fresh sample.
2. Use the test procedure to measure the concentration of each of the spiked samples. Start with the smallest sample spike. Measure each of the spiked samples in the instrument.
3. Compare the expected result to the actual result. The expected copper concentration increase is 0.3 mg/L for each 0.1 mL of standard added. For the AccuVac Ampul procedure, the expected copper concentration increase is 0.3 mg/L for each 0.2 mL of standard added.

### Standard solution method

Use the standard solution method to validate the test procedure, the reagents and the instrument.

Items to collect:

- Copper Standard Solution, 100-mg/L
- 100-mL volumetric flask, Class A
- 4-mL volumetric pipet, Class A and pipet filler
- Deionized water

1. Prepare a 4.00-mg/L copper standard solution as follows:
  - a. Use a pipet to add 4.00 mL of 100-mg/L copper standard solution into the volumetric flask.
  - b. Dilute to the mark with deionized water. Mix well. Prepare this solution daily.
2. Use the test procedure to measure the concentration of the prepared standard solution.
3. Compare the expected result to the actual result.

**Note:** The factory calibration can be adjusted slightly with the standard calibration adjust option so that the instrument shows the expected value of the standard solution. The adjusted calibration is then used for all test results. This adjustment can increase the test accuracy when there are slight variations in the reagents or instruments.

### Method performance

The method performance data that follows was derived from laboratory tests that were measured on a Pocket Colorimeter II during ideal test conditions. Users can get different results under different test conditions.

Precision (95% confidence interval)
1.00 ± 0.04 mg/L Cu

### Summary of method

Copper in the sample reacts with a salt of bicinchoninic acid contained in CuVer 1 or CuVer 2 Copper Reagent to form a purple colored complex in proportion to the copper concentration.

### Consumables and replacements items

#### Required reagents

Description	Quantity/test	Unit	Item no.
CuVer® 1 Copper Reagent Powder Pillow, 10-mL	1	100/pkg	2105869
OR			
CuVer® 2 Copper Reagent AccuVac® Ampul	1	25/pkg	2504025

**Required apparatus (powder pillows)**

Description	Quantity/test	Unit	Item no.
Sample cells, 10-mL round, 25 mm x 60 mm	2	6/pkg	2427606

**Required apparatus (AccuVac Ampul)**

Description	Quantity/Test	Unit	Item no.
Sample cell, 10-mL round, 25 mm x 60 mm	1	6/pkg	2427606
Beaker, 50-mL	1	each	50041H
Stoppers for 18-mm tubes and AccuVac Ampuls	2	6/pkg	173106

**Recommended standards**

Description	Unit	Item no.
Copper Standard Solution, 100-mg/L as Cu	100 mL	12842
Copper Voluette <sup>®</sup> Ampule Standard, 75-mg/L as Cu, 10-mL	16/pkg	1424710
Metals Drinking Water Standard, LR for Cu, Fe, Mn	500 mL	2833749
Metals Drinking Water Standard, HR for Cu, Fe, Mn	500 mL	2833649

**Optional reagents and apparatus**

Description	Unit	Item no.
AccuVac <sup>®</sup> Ampul Snapper	each	2405200
Mixing cylinder, graduated, 25-mL	each	2088640
Mixing cylinder, graduated, 50-mL	each	189641
Beaker, 50-mL	each	50041H
CuVer 2 Copper Reagent Powder Pillows	100/pkg	2188299
Flask, volumetric, Class A, 100-mL	each	1457442
Formaldehyde, ACS	100 mL MDB	205932
Filter paper, 2–3-micron, pleated, 12.5-cm	100/pkg	189457
Funnel, poly, 65-mm	each	108367
Nitric Acid, concentrated	500 mL	15249
Pipet, TenSette <sup>®</sup> , 0.1–1.0 mL	each	1970001
Pipet tips for TenSette <sup>®</sup> Pipet, 0.1–1.0 mL	50/pkg	2185696
Pipet tips for TenSette <sup>®</sup> Pipet, 0.1–1.0 mL	1000/pkg	2185628
Paper, pH, 1.0–11.0	5 rolls/pkg	39133
Potassium Chloride Solution, 50% saturated	25 mL	1429323
Potassium Hydroxide Solution, 8 N	100 mL MDB	28232H
Sample cells, 25-mL, matched, 1" square	2/pkg	2612602
AccuVac <sup>®</sup> Ampul Snapper	each	2405200
Ampule Breaker, 10-mL Voluette <sup>®</sup> Ampules	each	2196800
Sample cells, 25-mm round	6/pkg	2401906
Copper, Free and Total Reagent Set, includes:	each	2439200

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**Optional reagents and apparatus (continued)**

Description	Unit	Item no.
Hydrosulfite Reagent Powder Pillows	100/pkg	2118869
Copper, Free, Reagent Powder Pillows	100/pkg	2182369

**FOR TECHNICAL ASSISTANCE, PRICE INFORMATION AND ORDERING:**

In the U.S.A. – Call toll-free 800-227-4224

Outside the U.S.A. – Contact the HACH office or distributor serving you.

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