

## USEPA<sup>1,2</sup> Bicinchoninate Method<sup>3</sup>      Method 8506 (CuVer 1) and Method 8026 (CuVer 2) 0.04 to 5.00 mg/L Cu      Powder Pillows or AccuVac<sup>®</sup> Ampuls

**Scope and application:** For water, wastewater and seawater<sup>4</sup>; Method 8506 USEPA approved for reporting wastewater analysis (digestion required)<sup>5</sup>

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

<sup>2</sup> Hach Method 8026 is USEPA approved for the determination of copper in drinking water, Federal Register Volume 81, Number 138 (Tuesday, July 19, 2016).

<sup>3</sup> Adapted from Nakano, S., Yakugaku Zasshi, 82 486-491 (1962) [Chemical Abstracts, 58 3390e (1963)].

<sup>4</sup> Pretreatment required for the powder pillow method- refer to the Interference section.

<sup>5</sup> Federal Register, 45 (105) 36166 (May 29, 1980).





### Test preparation

### Instrument-specific information



Table 1 shows sample cell and orientation requirements for reagent addition tests, such as powder pillow or bulk reagent tests. Table 2 shows sample cell and adapter requirements for AccuVac Ampul tests. The tables also show all of the instruments that have the program for this test.

To use the table, select an instrument, then read across to find the applicable information for this test.

**Table 1 Instrument-specific information for reagent addition**

Instrument	Sample cell orientation	Sample cell
DR 6000 DR 3800 DR 2800 DR 2700 DR 1900	The fill line is to the right.	2495402 
DR 5000 DR 3900	The fill line is toward the user.	
DR 900	The orientation mark is toward the user.	2401906 

**Table 2 Instrument-specific information for AccuVac Ampuls**

Instrument	Adapter	Sample cell
DR 6000 DR 5000 DR 900	—	2427606 
DR 3900	LZV846 (A)	
DR 1900	9609900 or 9609800 (C)	
DR 3800 DR 2800 DR 2700	LZV584 (C)	2122800 

## Before starting

Install the instrument cap on the DR 900 cell holder before ZERO or READ is pushed.

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.

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 automatically with the reagent blank adjust option.

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. (For information about sample cells, adapters or light shields, refer to <a href="#">Instrument-specific information</a> on page 1.)	2

Refer to [Consumables and replacements items](#) on page 7 for order information.

### AccuVac Ampuls

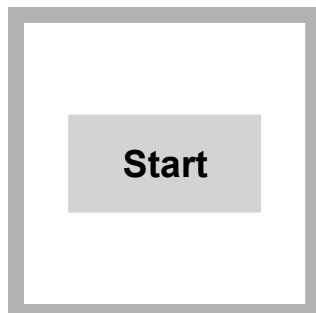
Description	Quantity
CuVer <sup>®</sup> 2 Reagent AccuVac <sup>®</sup> Ampul	1
Beaker, 50-mL	1
Sample cells (For information about sample cells, adapters or light shields, refer to <a href="#">Instrument-specific information</a> on page 1.)	1
Stopper for 18-mm tubes and AccuVac Ampuls	1

Refer to [Consumables and replacements items](#) on page 7 for order information.

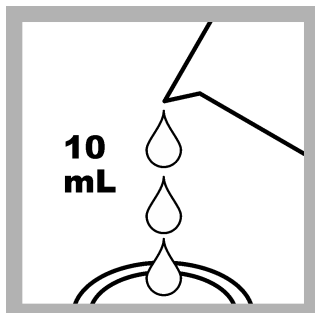
## Sample collection and storage

- 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.

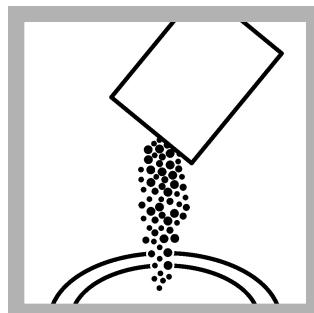
## Powder pillow procedure (Method 8506)



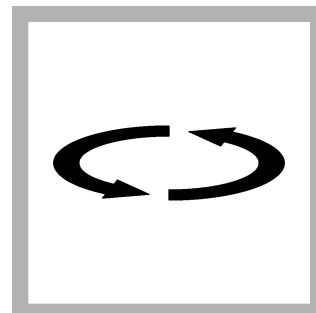
1. Start program **135 Copper, Bicin**. For information about sample cells, adapters or light shields, refer to [Instrument-specific information](#) on page 1.



2. **Prepare the sample:** Fill a sample cell with 10 mL of sample.



3. Add the contents of one CuVer 1 Copper Reagent powder pillow.

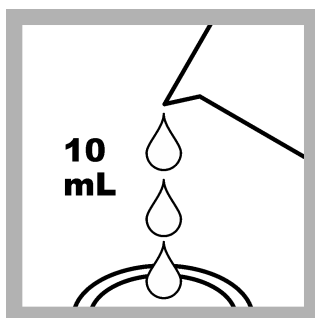


4. Swirl to mix.

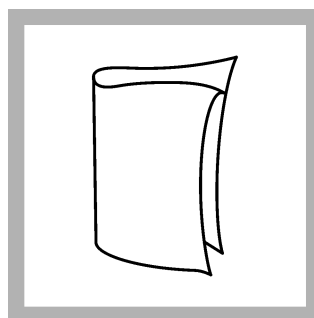


5. Start the instrument timer. A 2-minute reaction time starts.

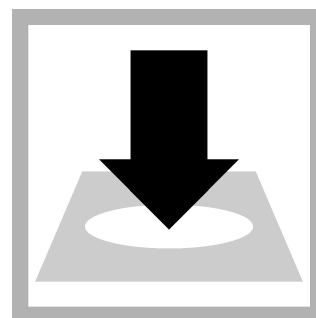
The sample shows a purple color when copper in the sample mixes with the reagent powder. Undissolved powder does not affect accuracy.



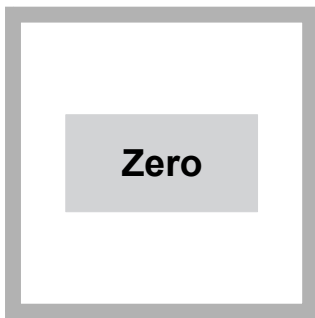
6. **Prepare the blank:** Fill a second sample cell with 10 mL of sample.



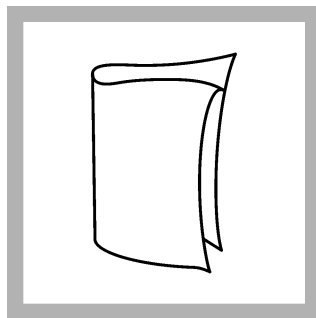
7. Clean the blank sample cell.



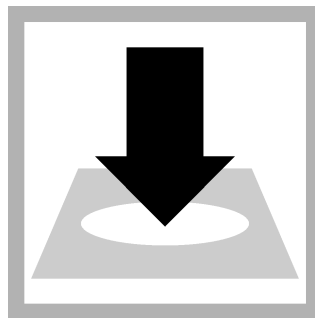
8. Insert the blank into the cell holder.



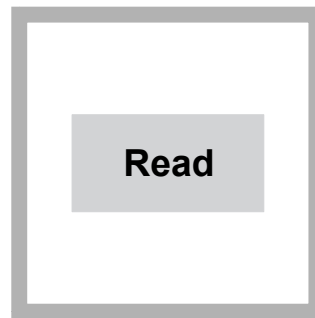
9. Push **ZERO**. The display shows 0.00 mg/L Cu.



10. Clean the prepared sample cell.

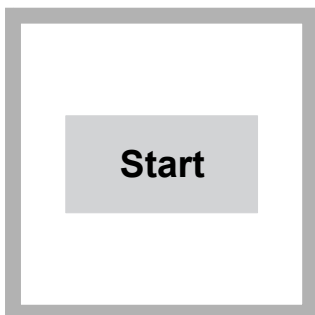


11. Within 30 minutes after the timer expires, insert the prepared sample into the cell holder.

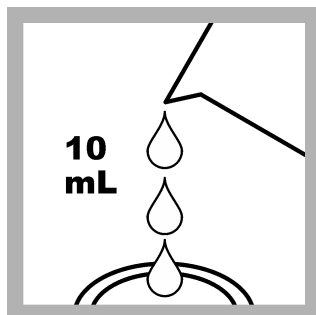


12. Push **READ**. Results show in mg/L Cu.

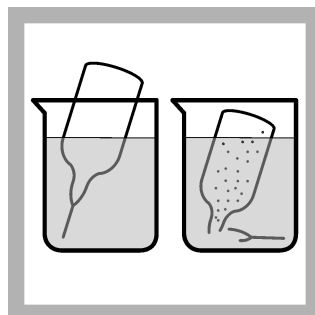
### AccuVac Ampul procedure (Method 8026)



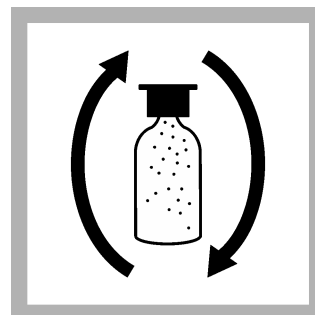
1. Start program **140 Copper, Bicin. AV**. For information about sample cells, adapters or light shields, refer to [Instrument-specific information](#) on page 1.



2. **Prepare the blank:** Fill the sample cell with 10 mL of sample.



3. **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.

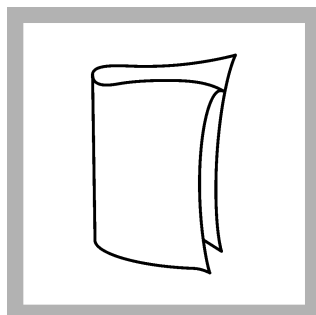


4. Quickly invert the AccuVac Ampul several times to mix.

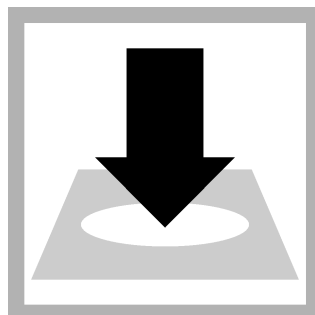


5. Start the instrument timer. A 2-minute reaction time starts.

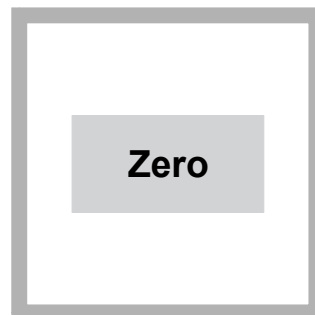
The sample shows a purple color when copper in the sample mixes with the reagent powder. Undissolved powder does not affect accuracy.



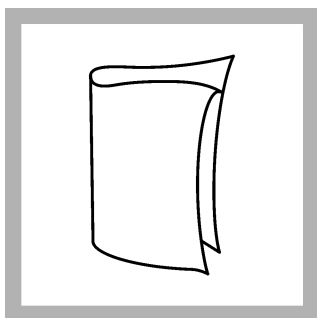
6. When the timer expires, clean the blank sample cell.



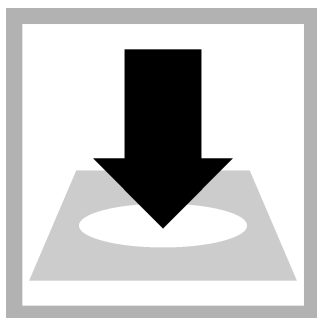
7. Insert the blank into the cell holder.



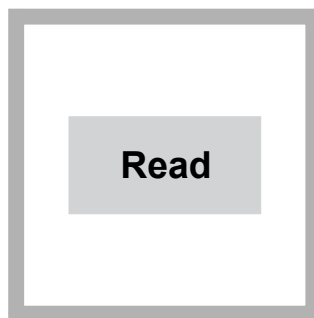
8. Push **ZERO**. The display shows 0.00 mg/L Cu.



9. Clean the AccuVac Ampul.



10. Within 30 minutes of the reagent addition, insert the prepared sample AccuVac Ampul into the cell holder.



11. Push **READ**. Results show in mg/L Cu.

## Interferences

[Table 3](#) gives treatments for powder pillows. [Table 4](#) gives treatments for AccuVac Ampuls.

To differentiate free copper from that complexed to EDTA or other complexing agents, use the powder pillow procedure with the information that follows:

- Use a 25-mL sample and 25-mL sample cells.
- Add a Free Copper Reagent Powder Pillow for 25-mL samples in step 3. Do not add the CuVer 1 reagent. The test result is the mg/L free copper in the sample.
- After the read step, add a Hydrosulfite Reagent Powder Pillow to the same sample, wait 2 minutes and read the result. The test result after the addition of both reagents is total dissolved copper.
- To determine the mg/L of complexed copper, subtract the mg/L free copper from the mg/L total dissolved copper.

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 3 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 above 4, then start the test.
Aluminum, Al <sup>3+</sup>	Use the powder pillow procedure, but use a CuVer 2 Copper Reagent Powder Pillow and not the CuVer 1 Pillow. Results include total dissolved copper (free and complexed). Use a 25-mL sample volume.
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 use a CuVer 2 Copper Reagent Powder Pillow and not the CuVer 1 Pillow. Results include total dissolved copper (free and complexed). Use a 25-mL sample volume.
Iron, Fe <sup>3+</sup>	Use the powder pillow procedure, but use a CuVer 2 Copper Reagent Powder Pillow and not the CuVer 1 Pillow. Results include total dissolved copper (free and complexed). Use a 25-mL sample volume.
Silver, Ag <sup>+</sup>	If a turbidity remains and turns black, silver interference is likely. Add 20 drops of 50% saturated Potassium Chloride Solution to 75 mL of sample, then filter through a fine or highly retentive filter. Use the filtered sample in the test procedure.

**Table 4 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 above 4, then start the test.
Aluminum, Al <sup>3+</sup>	Reagents accommodate high levels.
Cyanide, CN <sup>-</sup>	Prevents full color development. Add 0.5 mL of formaldehyde per 25-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 (sample spike)

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

Items to collect:

- 100 mg/L Copper Standard Solution
- 5-mL volumetric pipet, Class A and pipet filler
- Mixing cylinder, 50 mL
- Deionized water
- Pipet, TenSette®, 0.1–1.0 mL and tips

1. Prepare a 12.5 mg/L copper standard solution as follows:
  - a. Use a pipet to add 5.00 mL of a 100 mg/L copper standard solution into a 50-mL mixing cylinder.
  - b. Dilute to the 40-mL mark with deionized water. Mix well. Prepare this solution daily.
2. Use the test procedure to measure the concentration of the sample, then keep the (unspiked) sample in the instrument.
3. Go to the Standard Additions option in the instrument menu.
4. Select the values for standard concentration, sample volume and spike volumes.
5. Prepare three spiked samples: use the TenSette pipet to add 0.1 mL, 0.2 mL and 0.3 mL of the prepared standard solution, respectively, to three 10-mL portions of fresh sample. Mix well.

**Note:** For AccuVac® Ampuls, add 0.2 mL, 0.4 mL and 0.6 mL of a 75 mg/L Copper Voluette Ampule Standard to three 50-mL portions of fresh sample.

6. 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.
7. Select **Graph** to compare the expected results to the actual results.

**Note:** If the actual results are significantly different from the expected results, make sure that the sample volumes and sample spikes are measured accurately. The sample volumes and sample spikes that are used should agree with the selections in the standard additions menu. If the results are not within acceptable limits, the sample may contain an interference.

### 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 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 small variations in the reagents or instruments.

## Method performance

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

Program	Standard	Precision (95% Confidence Interval)	Sensitivity Concentration change per 0.010 Abs change
135	1.00 mg/L Cu	0.97–1.03 mg/L Cu	0.04 mg/L Cu
140	1.00 mg/L Cu	0.97–1.03 mg/L Cu	0.03 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. The measurement wavelength is 560 nm.

## Consumables and replacements items

### Required reagents

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

### Required apparatus

Description	Quantity/test	Unit	Item no.
AccuVac Snapper	1	each	2405200
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® 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.
Beaker, 50 mL	each	50041H
CuVer 2 Copper Reagent Powder Pillows	100/pkg	2188299
Mixing cylinder, graduated, 50 mL	each	189641
Formaldehyde, ACS	100 mL MDB	205932
Nitric Acid, concentrated	500 mL	15249
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® Ampul Snapper	each	2405200
Ampule Breaker, 10-mL Voluette® Ampules	each	2196800
Sample cells, 25-mm round	6/pkg	2401906
Copper, Free and Total Reagent Set, includes:	each	2439200
Hydrosulfite Reagent Powder Pillows	100/pkg	2118869
Free Copper Reagent Powder Pillows, 25 mL	100/pkg	2118669



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