

Standard range: 0.03 to 3.0 mg/L Cu

Method  
EZ1010



## Test preparation

### Before starting

Chemical exposure hazard. Obey laboratory safety procedures and wear all of the personal protective equipment appropriate to the chemicals that are handled. Refer to the current safety data sheets (MSDS/SDS) for safety protocols.

Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment. Dispose of chemicals and wastes in accordance with local, regional and national regulations.

Review the Safety Data Sheets (MSDS/SDS) before the bottles are filled or the reagents are prepared.

All chemicals must be of reagent grade, ACS grade or better<sup>1</sup>. The use of pro-analysis chemicals is recommended. Use of reagents that are not of sufficient quality can have a negative effect on the analyzer performance.

All EZ analyzers are put through long tests with standard solutions, reagents and dilution water prepared with Type I water or better water as specified in ASTM D1193-91.

To get the specifications shown on the data sheet, method and reagents sheet and acceptance test reports, the same water quality (or better) must be used to prepare the standard solutions, reagents and dilution water.

In addition, prepare the standard solutions for an EZ analyzer with water that does not contain the parameter to be measured or interferences for the method.

When operating the device, always make sure to follow the reagent recommendations given in [Reagent consumption](#) on page 3.

For longer-term storage, keep the reagents in a cold and dark place. Do not keep reagents longer than recommended. If applicable, keep the reagents in a refrigerator during measurements. Refer to [Reagent consumption](#) on page 3 for the reagent temperature.

The manufacturer recommends to replace the reagents, stock and standard solution at 28-day intervals unless specified differently in the sections that follow. Do not mix used reagents with freshly prepared reagents. If reagents, standards or DI water in the containers are replaced, discard all of the container contents in accordance with local, regional and national regulations. Rinse out all of the containers and then fill each container with freshly prepared new reagent.

### Specifications

Specifications are subject to change without notice.

Specification	Details
Analysis method	Bicinchoninate colorimetric method
Measurement wavelength	546 nm
Parameter	Long description: Copper Short description (default): Cu Options: None
Unit	mg/L (default); ppm, ppb, µg/L
Precision	The precision value is found on the full-scale range for standard test solutions. Refer to <a href="#">Table 1</a> .
Cleaning	Automatic or manual; frequency is freely programmable

<sup>1</sup> Analytical Reagent (AR), Guaranteed Reagent (GR), UNIVAR, AnalaR, Premium Reagent (PR), ReagentCertified ACS reagent, ACS Plus reagent, puriss p.a. ACS reagent, ReagentPlus®, TraceCERT®, Suprapur®, Ultrapur®, or better are also possible.

Specification	Details
Calibration	Automatic or manual; 2-point, offset or slope; frequency is freely programmable <i>Note: The manufacturer recommends that a calibration is done when the reagents are replaced.</i>
Validation	Automatic or manual; frequency is freely programmable
Interferences	Acidity, hardness does not interfere < 400 mg/L (as CaCO <sub>3</sub> ), metal ions, e.g., aluminum (III) (Al <sup>3+</sup> ) > 10 mg/L, cyanide (CN <sup>-</sup> ), iron(III) (Fe <sup>3+</sup> ) > 10 mg/L, nickel (II) (Ni <sup>2+</sup> ) and silver (II) (Ag <sup>+</sup> ). Large quantities of color and turbidity interfere. Fats, oil, proteins, surfactants and tar interfere.

**Table 1 Measuring ranges**

Range code	Description	LOD (mg/L)	Range (mg/L)	Precision (%)	Cycle time (minutes)	
					Continuous	Default
A	10% of standard range	0.003	0.3	2	6	10
B	25% of standard range	0.02	0.75	2	6	10
C	50% of standard range	0.02	1.5	2	6	10
<b>0</b>	<b>Standard range</b>	<b>0.03</b>	<b>3</b>	<b>2</b>	<b>6</b>	<b>10</b>
V	internal dispenser dilution (factor 5)	0.15	15	2	9	15
W	internal dispenser dilution (factor 10)	0.3	30	2	9	15
X	internal dispenser dilution (factor 25)	0.75	75	2	9	15
Y	internal dispenser dilution (factor 50)	1.5	150	2	9	15
Z	internal dispenser dilution (factor 75)	2.25	225	2	9	15
5	internal dispenser dilution (factor 100)	3	300	2	9	15

## Summary of method

### Summary

Copper ions in the water sample react with 2,2'-biquinoline at a pH between 6 and 8 to form a strong pink color. The absorbance is measured at a wavelength of 546 nm.

### Analysis steps

The analysis vessel is rinsed and filled with new sample. The acid reagent is added and the initial absorbance value is measured. The color reagent is then added and a stir period starts.

After the stir period, the color is fully developed and the final absorbance value is measured. The analyzer uses the absorbance values and Beer's Law to calculate the concentration of copper in the sample.

### Calibration

The calibration procedure measures the REF1 solution (Channel 9, REF1 valve) and the REF2 solution (Channel 10, REF2 valve).

### Validation

The validation procedure measures the REF2 solution (Channel 10, REF2 valve).

## Reagent consumption

Table 2, Table 3 and Table 4 show the consumption rate of the reagents and calibration standards. Examine the consumption of the reagents after 28 days to adjust the quantities prepared. Refer to [Necessary reagents](#) on page 4 to collect the necessary items to prepare the reagents.

**Table 2 Reagent consumption**

Product information			Consumption		Recommendation		
Code	Label	Product	Each analysis	Per 28 days, rate of 1 analysis/10 minutes	Use life	Containers	Operation temperature
Red	Reagent 1	Acid	~ 1 mL	~ 4.1 L	28 days	Plastic; 5 L	10 to 30 °C (50 to 86 °F)
Blue	Reagent 2	Color	~ 1 mL	~ 4.1 L	28 days	Plastic; 5 L	10 to 30 °C (50 to 86 °F)

**Table 3 Calibration standards**

Product information		Consumption	Recommendation	
Label	Product	Per calibration	Use life	Containers
REF1	REF1 standard	~ 0.2 L	28 days	Plastic, 1 L (align with recommendation)
REF2	REF2 standard	~ 0.2 L	28 days	Plastic, 1 L (align with recommendation)

**Table 4 Calibration recommendations**

Calibration	Time (minutes)		Recommended frequency	Solutions
	No dilution	With dilution		
Offset	18	26	—	REF1
2-point (recommended)	36	52	Reagent replacement (28 days)	REF1 and REF2

## DI water consumption

The volumes shown in Table 5 are an estimation of the consumption for rinse and dilution water based on a standard operating procedure as given in the specifications of the EZ analyzer.

**Note:** Rinse water volumes can increase because of the sample matrix.

**Note:** The range codes A, B, C, 0 are configured as default without the use of rinse and dilution water.

**Table 5 DI water consumption**

Range code	Rinse water Type I (mL/analysis)	Dilution water Type I (mL/analysis)	Total (mL/analysis)	Per 28 days, rate of 1 analysis each 15 minutes
A - B - C - 0 (no dilution)	—	—	—	—
V - W - X - Y - Z - 5 (with dilution)	51 mL	16 mL	67 mL	180 L

## Rinse water

If the analyzer does a dilution, a deionized water rinse must be used. If no dilution is done, use the sample to rinse. If there is a filter panel in front of the analyzer, make sure that the rinse water also flows through the filter.

## Necessary reagents

A reagent kit with mixing instructions is available that decreases the preparation time. Refer to [Table 6](#). The full list of reagents is shown in [Table 7](#). The product name, formula, molecular weight, CAS number and the necessary quantity to prepare 1 L of the reagents are given.

**Table 6 Reagent kit for EZ1010: APPC1010KTO-X<sup>2</sup>**

Code	Label	Product	Volume	Item no.
Red	Reagent 1	Acid	2 L (2x)	APPC101001-X <sup>2</sup>
Blue	Reagent 2	Color	2 L (2x)	APPC230201-X <sup>2</sup>
—	Stock solution	1000 mg/L Cu	100 mL (1x) <sup>3</sup>	259342

**Table 7 Reagent list**

Solutions	Products	Formula	MW (g/mol)	CAS number	For each 1 L solution
Reagent 1: Acid Code: Red	Nitric acid (65%)	HNO <sub>3</sub>	63.01	7697-37-2	35 mL
Reagent 2: Color Code: Blue	2,2'-Biquinoline-4,4'-dicarboxylic acid dipotassium salt trihydrate	C <sub>2</sub> OH <sub>10</sub> K <sub>2</sub> N <sub>2</sub> O <sub>4</sub> * 3H <sub>2</sub> O	474.55	207124-63-8	3 g
	Ammonium hydroxide solution 25% <sup>4</sup>	NH <sub>4</sub> OH	35.05	1336-21-6	50 mL
	Hydroxylamine hydrochloride	H <sub>4</sub> CINO	69.49	5470-11-1	1 g
Stock solution	Copper (II) sulfate pentahydrate	CuO <sub>4</sub> S * 5H <sub>2</sub> O	249.69	7758-99-8	3.9293 g
	Nitric acid (65%)	HNO <sub>3</sub>	63.01	7697-37-2	35 mL
REF1 calibration standard	Deionized water (Type I or better)	—	—	—	—
REF2 calibration standard	1000 mg/L Cu stock solution	—	—	—	Refer to <a href="#">Table 8</a> on page 6.
	Nitric acid (1 M)	HNO <sub>3</sub>	63.01	7697-37-2	8 mL
Validation standard (optional)	REF2 calibration standard	—	—	—	Refer to <a href="#">Validation standard</a> on page 6.
Cleaning solution (optional)	Hydrochloric acid (36%)	HCl	36.46	7647-01-0	41.5 mL

## Reagent preparation

As an alternative to the reagent kit, the user can prepare the reagents as follows. Refer to [Table 7](#) on page 4 to collect the applicable items. To calculate the correct reagent quantity, refer to [Reagent consumption](#) on page 3.

Make sure to discard the remaining solution from the analyzer bottles before new reagents are added.

<sup>2</sup> X = region code, e.g., EU

<sup>3</sup> More than 100 mL stock solution is necessary to prepare the REF2 calibration standard for the highest ranges.

<sup>4</sup> Density: 0.91 g/mL (20 °C)

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### Reagent 1: Acid

1. Add 500 mL of deionized water to a beaker.
2. Slowly mix in 35 mL nitric acid (HNO<sub>3</sub>, 65%).
3. Pour the solution into a 1000-mL volumetric flask.
4. Add deionized water to the mark.
5. Fully mix the solution.

**Note:** The concentration of the prepared solution is 0.5 M HNO<sub>3</sub>.

### Reagent 2: Color

1. Add 100 mL of deionized water to a beaker.
2. Add 3.0 g of 2,2'-biquinoline-4,4'-dicarboxylic acid dipotassium salt trihydrate (C<sub>20</sub>H<sub>10</sub>K<sub>2</sub>N<sub>2</sub>O<sub>4</sub> · 3H<sub>2</sub>O).
3. Mix until fully dissolved.
4. Add 50 mL of ammonium hydroxide solution (NH<sub>4</sub>OH, 25%) and fully mix.
5. In a different container, add 1.0 g of hydroxylamine hydrochloride to 100 mL of deionized water.
6. Mix until fully dissolved.
7. Mix the two solutions together.
8. Pour the solution into a 1000-mL volumetric flask.
9. Add deionized water to the mark.
10. Fully mix the solution.

### Calibration standards

Calibrations are completed with two standards: a REF1 calibration standard and a REF2 calibration standard. The REF2 calibration standard is a dilution of a stock solution.

### Stock solution

Prepare a 1000 mg/L Cu stock solution as follows. Refer to [Necessary reagents](#) on page 4 to collect the applicable items.

1. Add 500 mL of deionized water to a beaker.
2. Add 3.9293 g copper (II) sulfate pentahydrate (CuSO<sub>4</sub> · 5H<sub>2</sub>O).
3. Mix until fully dissolved.
4. Slowly add 35 mL of concentrated nitric acid (HNO<sub>3</sub>, 65%).
5. Fully mix the solution.
6. Pour the solution into a 1000-mL volumetric flask.
7. Add deionized water to the mark.
8. Fully mix the solution.

### REF1 calibration standard

Use deionized water for the REF1 calibration standard.

### REF2 calibration standard

Dilute the stock solution to prepare the REF2 calibration standard.

1. Use a pipet to add the applicable quantity (mL) of the stock solution into a 1000-mL volumetric flask. Refer to [Table 8](#).
2. Use a pipet to add the applicable quantity (mL) of 1 M nitric acid to the volumetric flask. Refer to [Table 8](#).
3. Add deionized water to the mark.
4. Fully mix the solution.

**Table 8 Calibration standard preparation**

Range code	REF2 concentration (mg/L Cu)	Quantity (mL) of stock solution	Quantity (mL) of 1 M HNO <sub>3</sub>
A	0.3	0.3	8
B	0.75	0.75	8
C	1.5	1.5	8
0	3	3	8
V	15	15	8
W	30	30	8
X	75	75	8
Y	150	150	8
Z	225	225	8
5	300	300	8

## Validation standard

By default, the automatic validation procedure is not enabled. When enabled, the default validation standard is the REF2 calibration standard. For best results, use a different standard solution from a different source for the validation standard. The concentration of the validation standard must be within the measuring range of the analyzer.

Before validation, connect the REF2 sample line to the validation standard. After validation, connect the REF2 sample line to the REF2 calibration standard again. For multi-channel setups, a different channel can be used.

## Cleaning solution

By default, the automatic cleaning procedure is not enabled. When enabled, the default volume of cleaning solution that is used during each cleaning cycle is 30 mL.

The cleaning procedure must prevent the collection of chemicals in the analyzer. For an accurate cleaning procedure, examine the cleaning solution and the cleaning interval for each application. Make sure that the cleaning procedure is sufficient. Change the cleaning procedure if necessary.

The manufacturer recommends to use a 0.5 M hydrochloric acid (HCl) solution. Refer to [Necessary reagents](#) on page 4. Prepare the solution as given in the steps that follow or use a commercially available solution.

1. Add 500 mL of deionized water to a beaker.
2. Slowly mix in 41.5 mL of concentrated hydrochloric acid (HCl, 37%).
3. Pour the solution into a 1000-mL volumetric flask.
4. Add deionized water to the mark.
5. Fully mix the solution.



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