



TECHNICAL MANUAL

Intracellular Cuprous Ion (Cu^+) Fluorometric Assay Kit

- **SKU CODE:** MAES0539
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Fluorometric
- **RUO:** Research-Use-Only

Intracellular Cuprous Ion (Cu⁺) Fluorometric Assay Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features	3
2. Storage & Expiry	3
3. Product Description	3
4. Kit Contents	4
5. Important Notes	5
6. Assay Procedure	5
7. Typical Data	8

1. Key Features

Specification:

96T

Measuring instrument:

Flow cytometer, fluorescence microscope

Sample type:

Cultured cells (suspension and adherent cells)

2. Storage & Expiry

This product should be stored at room temperature, protected from light, for up to 12 months. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

This kit can be used to measure intracellular cuprous ion (Cu^+) levels.

Cuprous ions (Cu^+) are essential trace elements for living organisms and participate in vital biological processes including energy metabolism and antioxidant defense. However, excess free intracellular Cu^+ binds to lipoylated proteins, triggering their oligomerization and functional loss, thereby inducing proteotoxic stress and ultimately leading to cuproptosis. Accordingly, the concentration of free intracellular Cu^+ must be tightly maintained at an extremely low level. Monitoring the dynamic homeostasis of intracellular Cu^+ is therefore critical for sustaining normal cellular functions.

This kit utilizes a specific fluorescent cuprous probe to quantify intracellular Cu^+ levels. The probe itself exhibits weak intrinsic fluorescence. Upon specific coordination with Cu^+ , the molecule undergoes a conformational change, resulting in a marked increase in fluorescence intensity.

4. Kit Contents

No	Component Name	Size (96T)	Storage
1	1 mmol/L Probe Solution	0.275 mL × 1 vial	RT, 12 months, protected from light

Note: All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.

Additional materials required:

1. Flow cytometer or fluorescence microscope.
2. Incubator (37°C).
3. 1X PBS (0.01 M, pH 7.4).

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

6. Assay Procedure

Detection of cultured cell samples

Instrument	Parameter
Flow cytometry	PE-Texas Red channel
Fluorescence microscope	Confocal microscope: Excitation (Ex): 480–500 nm; Emission (Em): 610–650 nm Conventional fluorescence microscope: PE filter set

A. Suspension Cells

1. Collect cells by centrifugation and discard the supernatant medium, then resuspend the cell pellet with PBS. Set up a blank control group (without probe) and experimental groups (probe-treated, customized according to research purposes, including drug treatment groups, positive control groups, etc.), with a minimum of three replicate wells per group. Adjust the cell density to no less than 1×10^5 cells/mL; for reference, resuspend 1×10^5 cells in 1 mL PBS. Pipette the cell suspension into corresponding wells of a 96-well cell culture plate at 0.2 mL per well.
2. Blank control wells: add 1 μ L culture medium.
Experimental wells: add 1 μ L probe solution.
3. Incubate the samples at 25°C in the dark for 5–20 min. The optimal staining duration varies among cell types. Start with an initial incubation time of 5 min, and optimize the incubation duration according to the cell line used to achieve optimal staining performance.

Detection – Flow cytometry

1. The loading cell number shall be no less than 1×10^4 cells. Detect the samples using the PE-Texas Red channel on the flow cytometer.

B. Adherent Cells

1. Seed cells into wells of a 24-well cell culture plate. Establish blank control groups (without probe) and test groups (supplemented with probe, customized according to experimental objectives, including drug treatment groups, positive control groups, etc.), with a minimum of three replicate wells for each group. The recommended seeding cell density is no less than 1×10^4 cells/mL. After cell seeding, incubate the plate overnight in a 37°C incubator with 5% CO₂.
2. Aspirate the culture medium, wash the cells twice with PBS, and discard the supernatant each time.
3. Add 0.5 mL PBS to each well.

4. Blank control wells: add 2.5 μ L culture medium.
Experimental wells: add 2.5 μ L probe solution.
5. Incubate the samples at 25°C in the dark for 5–20 min. The optimal staining duration varies among cell types. Start with an initial incubation time of 5 min, and optimize the incubation duration according to the cell line used to achieve optimal staining performance.

Detection – Flow cytometry

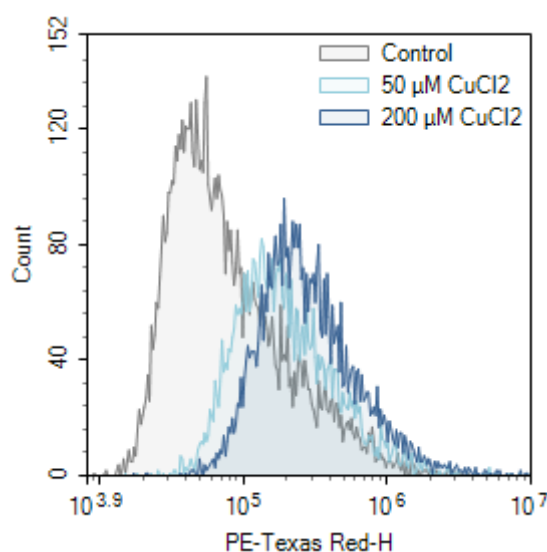
1. After staining, aspirate the supernatant from each well.
2. Add 0.2 mL trypsin to digest the adherent cells.
3. When digestion finishes, add 0.4 mL complete medium to terminate digestion.
4. Prepare cell suspension for flow cytometry; collect a minimum of 1×10^4 cells per sample.
5. Acquire fluorescence signals using the PE-Texas Red channel on the flow cytometer.

Detection – Fluorescence microscopy

1. After staining, aspirate the medium and wash the cells twice with PBS, discarding the supernatant after each wash.
2. Add fresh PBS again and observe cells under a fluorescence microscope.
3. Confocal microscope parameters: Excitation (Ex): 480–500 nm; Emission (Em): 610–650 nm.
4. A PE filter set is used for conventional fluorescence microscopy.

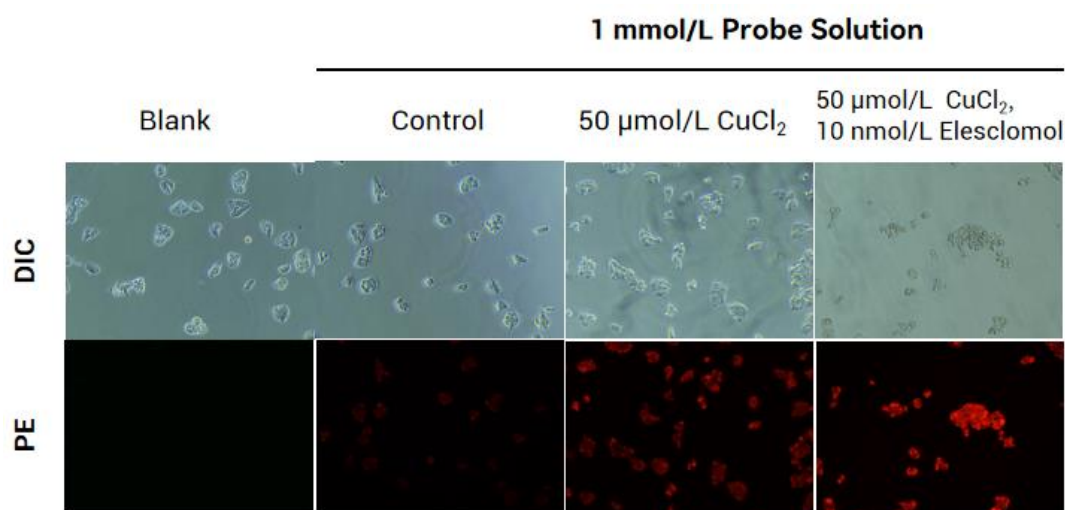
7. Typical Data

Flow cytometry results of 293T cells



293T cells were seeded at a density of 1×10^5 cells/mL and incubated overnight at 37°C under 5% CO₂. After aspiration of the original medium, cells were treated with medium containing 0, 50 $\mu\text{mol/L}$ or 200 $\mu\text{mol/L}$ copper chloride for 24 h before staining, and an equal volume of blank medium was supplemented to wells with 0 $\mu\text{mol/L}$ copper chloride. Fluorescence detection results demonstrated that the fluorescence signals in both 50 $\mu\text{mol/L}$ and 200 $\mu\text{mol/L}$ copper chloride groups were markedly elevated relative to the probe-only Control group, and fluorescence intensity increased with rising copper chloride concentrations.

Fluorescence microscopy results of HepG2 cells



HepG2 cells were seeded at a density of 1×10^5 cells/mL and incubated overnight at 37°C under 5% CO_2 . The cells were subjected to treatments with different conditions for another overnight incubation prior to staining. The results demonstrated that faint red fluorescence was observed in normal cells incubated with probe in the Control group, indicating the existence of endogenous Cu^+ within cells. After 18 h of treatment with 50 $\mu\text{mol/L}$ copper chloride, the intracellular Cu^+ level was elevated, accompanied by remarkably intensified red fluorescence after probe staining. Co-treatment with 10 nmol/L Elesclomol (Cu^{2+} ionophore) and 50 $\mu\text{mol/L}$ copper chloride for 18 h enabled Elesclomol to bind Cu^{2+} and efficiently facilitate cellular Cu^{2+} uptake, resulting in intracellular Cu^+ accumulation and further enhanced red fluorescence. Actual detection outcomes may vary depending on experimental conditions and testing instruments, and the fluorescence performance shown in the figure is for reference only.

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

