Electronic Supplementary Information

Single Fluorescent Probe for the Multiple Analyte Sensing: Efficient and Selective Detection of CN^-, HSO_3^- and extremely alkaline pH

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Contents:

presence of CN^- (35 μM) under different solvent conditions: (1) DMSO; (2) DMSO/H_2O, v/v, 4/1; (3) DMSO/H_2O, v/v, 3/2; (4) DMSO/H_2O, v/v, 2/3; (5) DMSO/H_2O, v/v, 1/4; (6) H_2O; (7) H_2O/CH_3CN, v/v, 4/1; (8) H_2O/CH_3CN, v/v, 3/2; (9) H_2O/CH_3CN, v/v, 2/3; (10) H_2O/CH_3CN, v/v, 1/4; (11) CH_3CN; λ_{ex} = 340 nm. Slits: 10 nm/5 nm. Voltage = 500 V.

Figures S5. Time-depended fluorescent ratio I_{376}/I_{575} changes of **IECBT** (10 μM) in the absence and present of CN^- (17 μM). λ_{ex} = 340 nm. Slits: 10 nm/5 nm. Voltage = 500 V.

Figures S6. Emission ratio I_{376}/I_{575} of **IECBT** (10 μM) in the presence of various anions (1 equiv. respectively) in DMSO. λ_{ex} = 340 nm. Slits: 10 nm/5 nm. Voltage = 500 V.

Figures S7. Changes in fluorescence spectra of **IECBT** (10 μM) at 568 nm in the absence and present of HSO_3^- (20 μM) in water as a function of pH. λ_{ex} = 465 nm. Slits: 10 nm/5 nm. Voltage = 600 V.

Figures S8. Time-depended fluorescent intensity changes at 568 nm of **IECBT** (10 μM) in the absence and present of HSO_3^- (10μM). λ_{ex} = 465 nm. Slits: 10 nm/5 nm. Voltage = 600 V.

Figures S9. The relative fluorescence intensity ratio $(F_0\text{-}F) / F_0$ of **IECBT** (10 μM) at 568 nm in the presence of various anions (1 equiv. respectively) in aqueous solution. λ_{ex} = 465 nm. Slits: 10 nm/5 nm. Voltage = 600 V.

Figures S10. Time-depended fluorescent intensity changes at 568 nm of **IECBT** (10 μM) at pH 6.44, 6.66, and 9.70, respectively. λ_{ex} = 465 nm. Slits: 10 nm/5 nm.

Voltage = 600 V.

Figures S11. Cell cytotoxic effect of **IECBT** on Hela cells. (1) control; (2) 0.1 μM; (3) 1 μM; (4) 10 μM. Data are expressed as mean values standard error of the mean of six independent experiments.

Figures S12. Cell cytotoxic effect of **IECBT** (10 μM) after upon addition of CN^- on Hela cells. CN^- concentrations were varied as following: (1) control; (2) 0.01 μM; (3) 0.1 μM; (4) 1 μM; (5) 5 μM; (6) 30 μM. Data are expressed as mean values standard error of the mean of six independent experiments.

1. The solvent dependence in the detection process of CN^-

The solvent of a system is often considered as a significant influencing factor on interactions. The effect of different solvent conditions on the fluorescence properties of the system was investigated (Figure S4). From Figure S4, we could find that **IECBT** was stable and displayed the best response for CN^- in DMSO. So, in the subsequent UV-vis and fluorescence experiments, DMSO was selected as a testing system to investigate the spectral response of **IECBT** to CN^-.

2. Cell cytotoxicity assay

An MTT assay was performed to test the cytotoxicity of **IECBT** as well as the cell viability after upon addition of CN^- on Hela cells. HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (fetal bovine serum), 100 $mg \cdot mL^{-1}$ penicillin, and 100 $\mu g \cdot mL^{-1}$ streptomycin in a 5% CO_2, water saturated incubator at 37 °C. Before the experiment, healthy HeLa cells (5×10^3) were plated into 96 well microtiter plates (Nunc) for 16 h, followed by the addition of different concentrations of **IECBT** (0 to 10 μM). The cells were then incubated

at 37 ℃ in an atmosphere of 5% CO_2 and 95% air for 24 h. After incubation, the solutions were aspirated and replaced by DMEM (180 μL), followed by the addition of 5 mg·mL^{-1} MTT solution (20 μL, final concentration of 0.5 mg·mL^{-1}) and incubated for 4 h. Unreacted dye was removed by aspiration; the insoluble formazan crystals were dissolved by adding dimethyl sulfoxide (200 μL) to each well and shaken for 10 min and measured spectrophotometrically in an ELISA reader at a wavelength of 490 nm. To evaluate the cytotoxicity of **IECBT** after upon addition of CN^- on Hela cells, the cells were also treated as previously described, except cells incubated with **IECBT** (10 μM) for 30 minutes were treated with a varying concentrations of CN^- (0, 0.01, 0.1, 1, 5, 30 μM) at 37 °C for 90 minutes. The cells were then washed with PBS (pH = 7.4), followed by analysis via MTT assays. Cells incubated with **IECBT** (10 μM) in a culture medium without CN^- were used as the control. Each group had six samples, and the spectrophotometer was calibrated to zero absorbance using culture medium without cells. The relative cell viability (%) related to the control groups was calculated as follows:

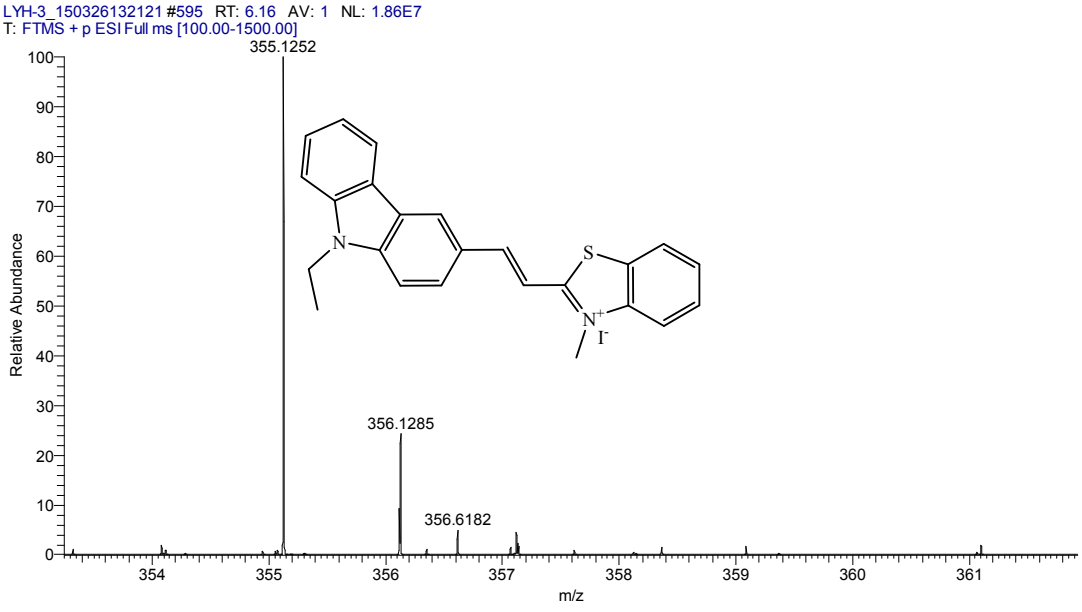
$$\text{Cell viability} = [A_{490}(\text{sample})/A_{490}(\text{control})] \times 100\ \%$$

Where A_{490} (sample) is the absorbance value of **IECBT** or **IECBT**-CN treated cells, and A_{490} (control) is the absorbance value of cells as control groups.

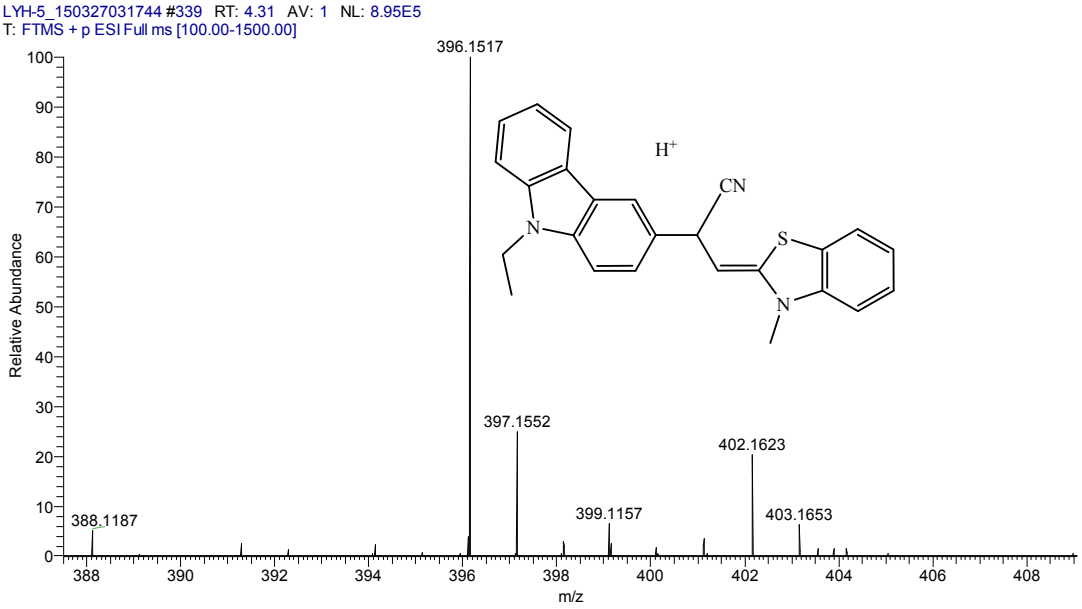
3. Culture of HeLa cells for intracellular imaging

To observe the subcellular distribution of **IECBT**, about 1×10^5 HeLa cells in growth medium (2 mL) were seeded on a 35 mm diameter round glass Petri dish and incubated for 48 h in a 5% CO_2 atmosphere. The medium was then removed. The cells were first incubated with **IECBT** (10 μM) dissolved in acetonitrile/water (4/6, v/v) for 30 min. The free **IECBT** was removed by washing the cells three times with PBS. The cells were then fixed with 4% paraformaldehyde (300 μL) for 8

minutes at room temperature and treated with DAPI (1 mg·mL^{-1}) for an additional 15 min. The medium was removed and the cells were rinsed with PBS (pH = 7.4) many times. Fluorescence images were collected on a ZEISS LSM 880 confocal laser scanning microscope with a 200× objective lens. DAPI was excited at 405 nm and its blue emission was collected in the 425-475 nm detection range; **IECBT** was excited at 488 nm and its red emissions were collected in the 500-600 nm detection range.



Figures S1. ESIMS analysis of **IECBT**.



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102 **Figures S2.** ESIMS analysis of **IECBT** in the present of CN^-.

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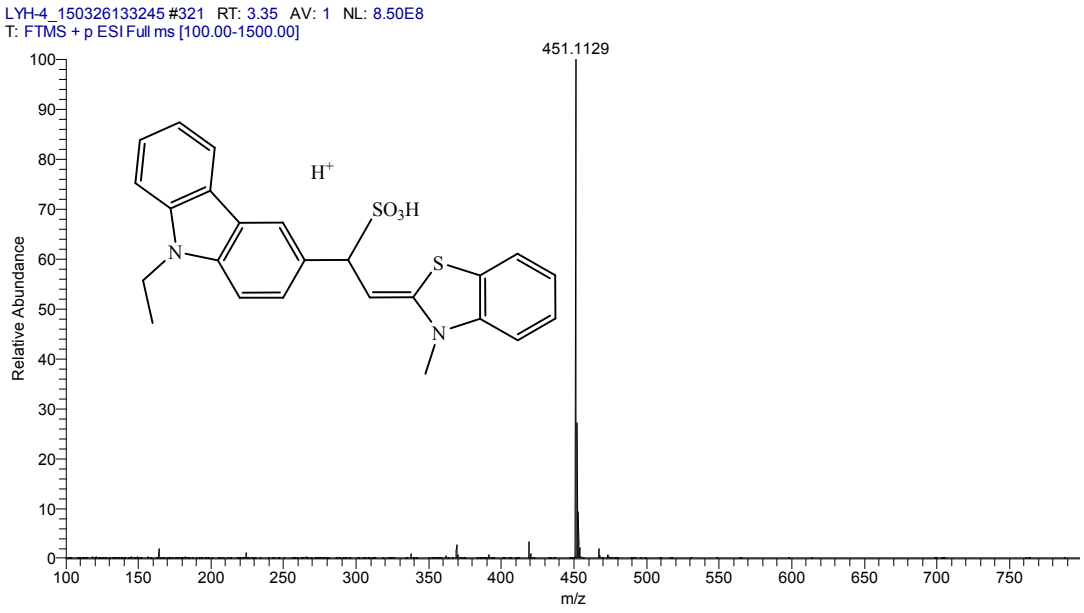

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106 **Figures S3.** ESIMS analysis of **IECBT** in the present of HSO_3^-.

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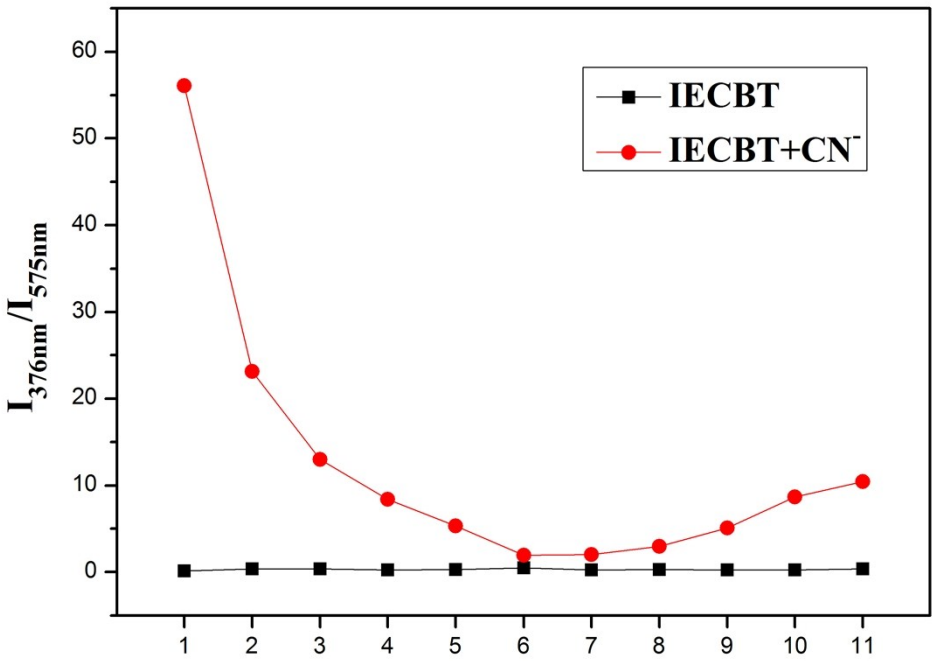

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110 **Figures S4.** Emission ratio I_{376}/I_{575} changes of **IECBT** (10 μM) in the absence or presence of CN^-

111 (35 μM) under different solvent conditions: (1) DMSO; (2) DMSO/H_2O, v/v, 4/1; (3) DMSO/H_2O,

112 v/v, 3/2; (4) DMSO/H_2O, v/v, 2/3; (5) DMSO/H_2O, v/v, 1/4; (6) H_2O; (7) H_2O/CH_3CN, v/v, 4/1;

113 (8) H_2O/CH_3CN, v/v, 3/2; (9) H_2O/CH_3CN, v/v, 2/3; (10) H_2O/CH_3CN, v/v, 1/4; (11) CH_3CN; λ_{ex}

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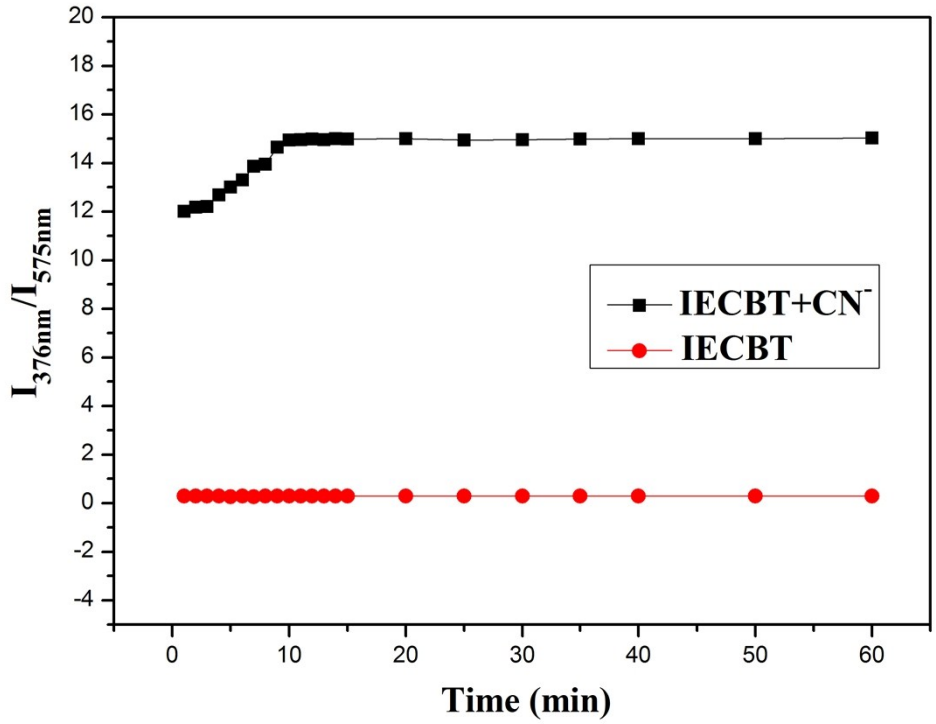

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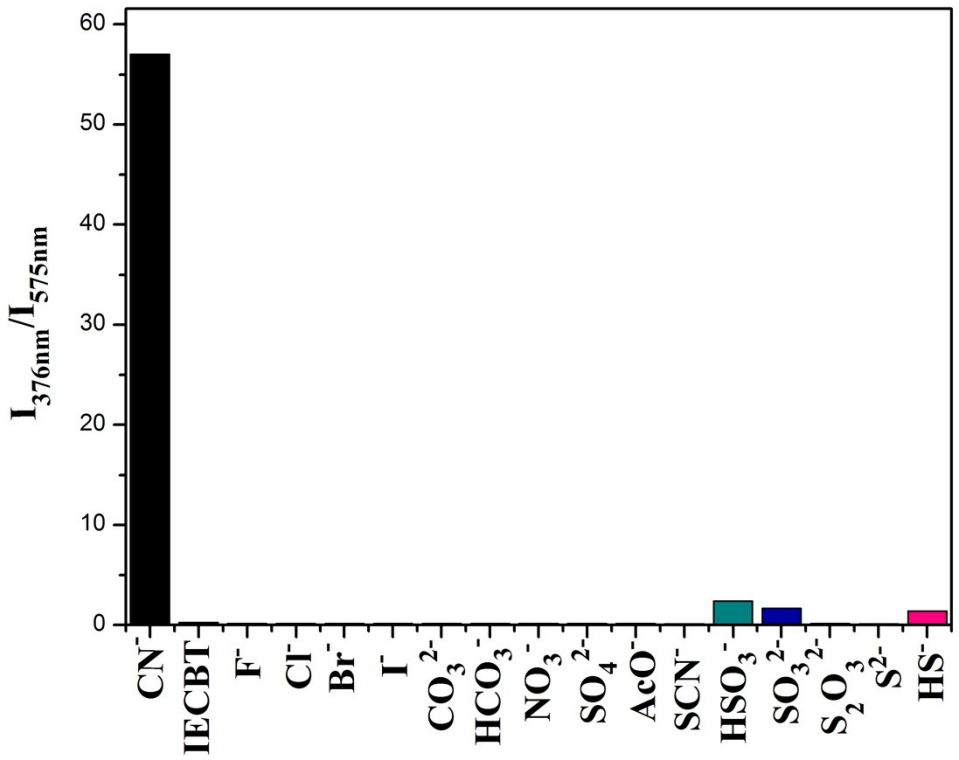

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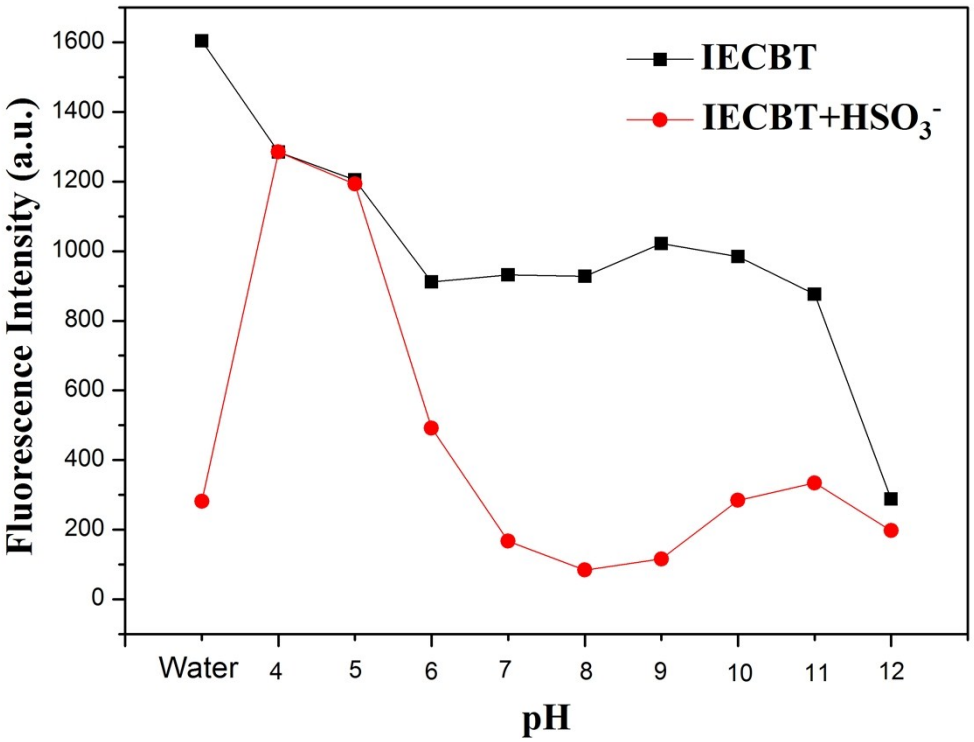

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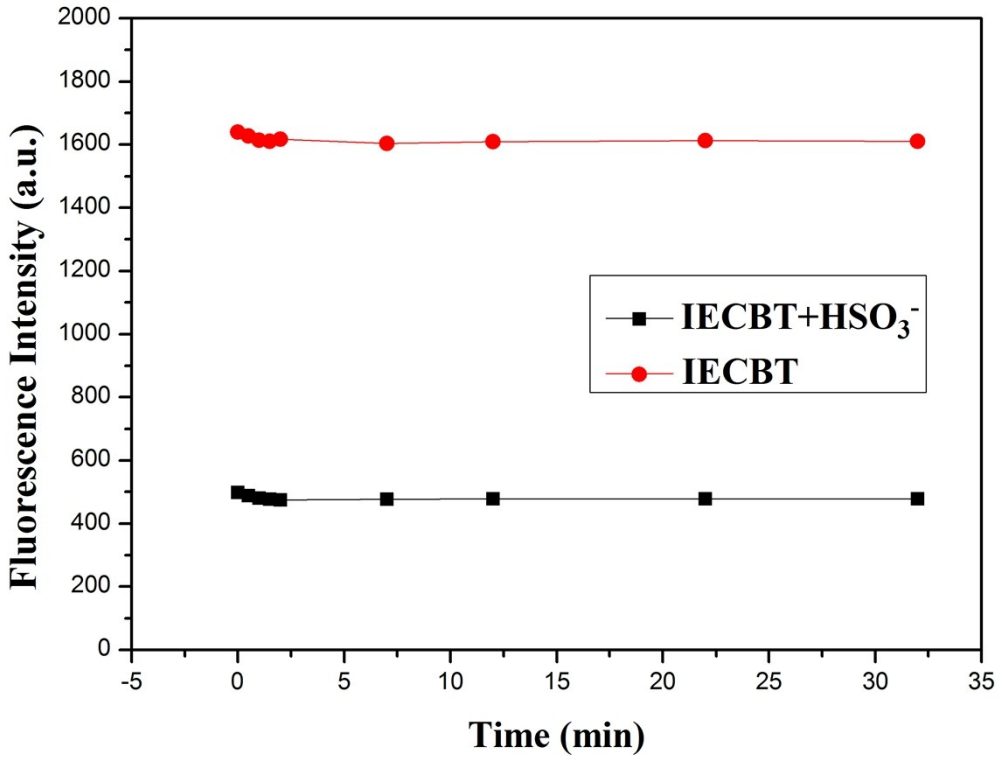

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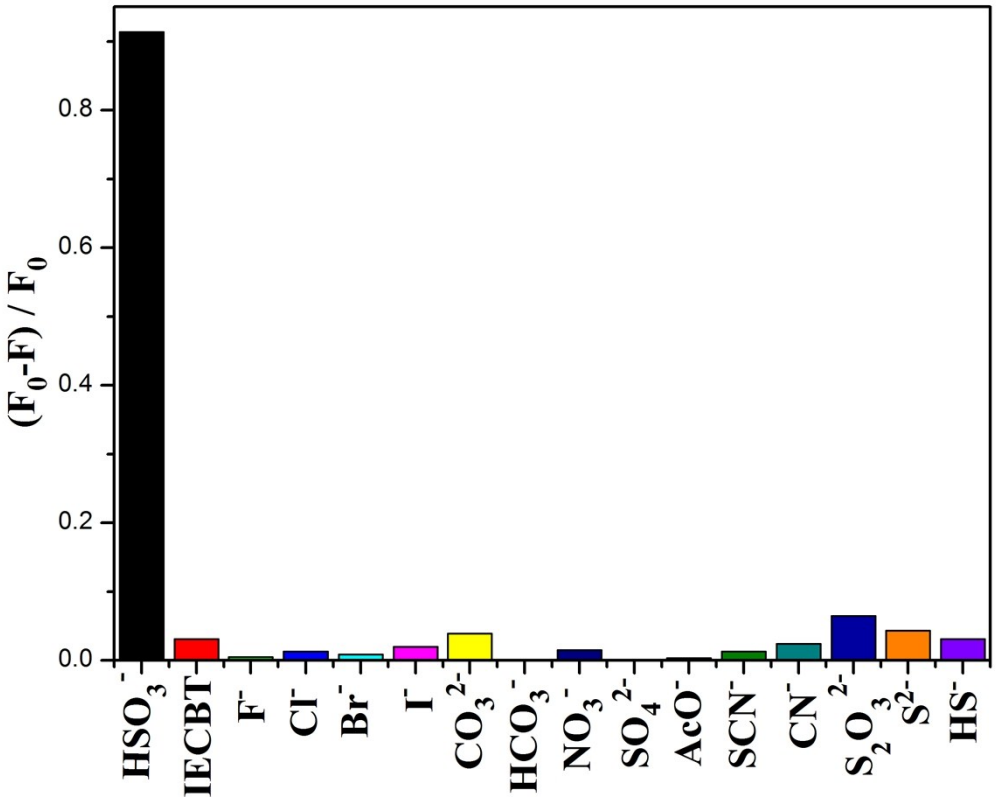

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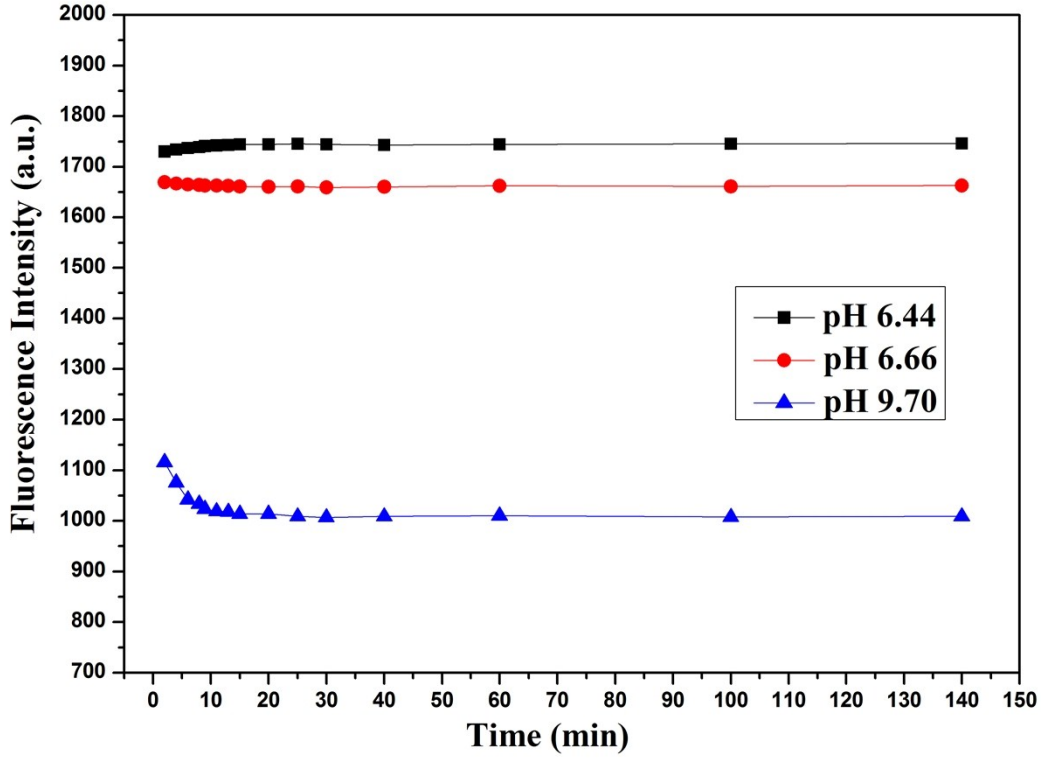

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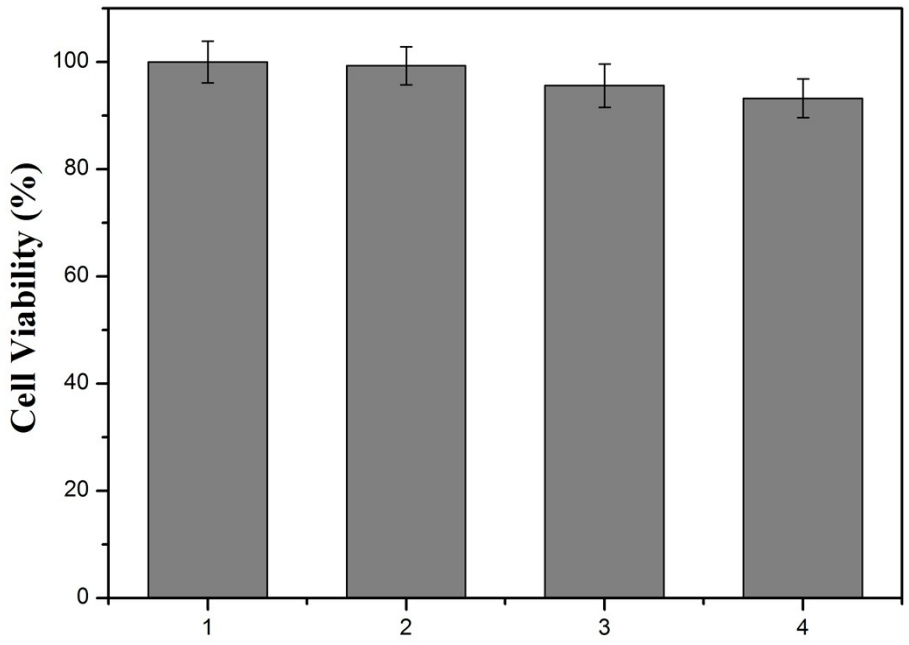

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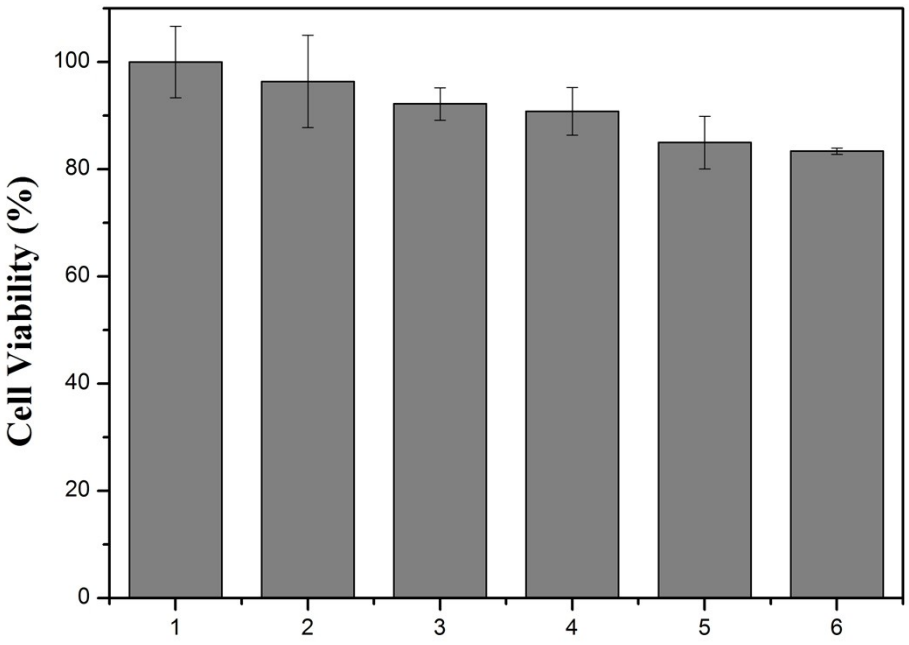

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