

Supplementary Information

Triple detection modes for Hg²⁺ sensing based on NBD-fluorescent and colorimetric sensor and its potential in cell imaging

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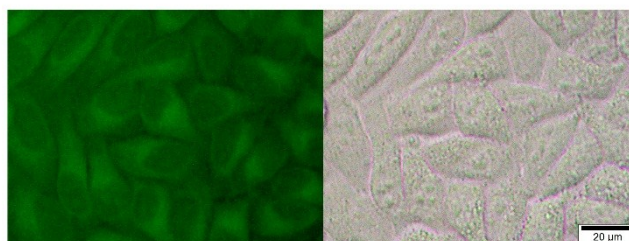
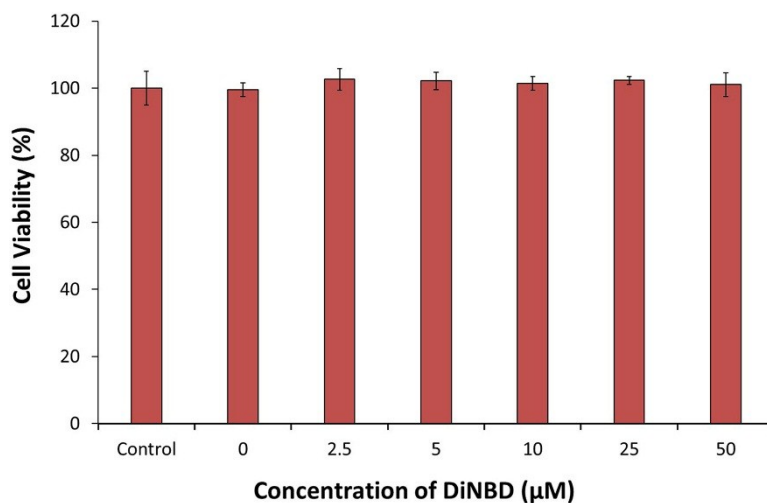
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Retaining of DiNBD within the cells after 24 h in growth media.

Fig. S1. Cell viability values (%) estimated by PrestobluTM Cell Viability reagent versus incubation concentrations of DiNBD. HeLa cells were incubated with DiNBD (2.5–50 µM) for 30 min (10% DMSO in PBS), and further cultured in growth media for 24 h before the cell viability assay. After 24 h culture, DiNBD remained within the cells but did not affect HeLa cell viability.

Cell viability assay

Cell survival of HeLa after treatment with **DiNBD** was measured using Prestobblue™ Cell Viability reagent (Invitrogen, USA) according to the manufacturer's protocol. In brief, cells were seeded in 96 well plate at 20 kcells/well and incubated overnight. Cells were treated with different concentrations of **DiNBD** (10% DMSO in phenol red free DMEM) ranging from 2.5–50 μ M, and two negative controls of DMEM alone or DMEM with 10% DMSO, then incubated for 30 min. Cells were washed twice followed by 24 h incubation in growth media. The media was replaced with 10% Prestobblue reagent in fresh media and incubated for 2 hours. Data values were measured as OD reading at 570 nm with reference subtraction at 600 nm. Data were expressed as mean \pm SD of triplicate experiments. Cell viability was calculated using the following formula: $100 \times [(OD_{570} \text{ of treated sample}) / (OD_{570} \text{ of untreated sample (DMEM alone)})]$. Cell morphology and retaining of **DiNBD** within the cells at the maximum concentration test of 50 μ M **DiNBD** was visualized under inverted phase contrast/fluorescence microscope (Olympus CKX53/ DP27-2, 100W mercury burner).