

## Supporting Information

### Ethylene glycol coated nanoceria protect against oxidative stress in human lens epithelium

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#### EGCNPs Characterisation

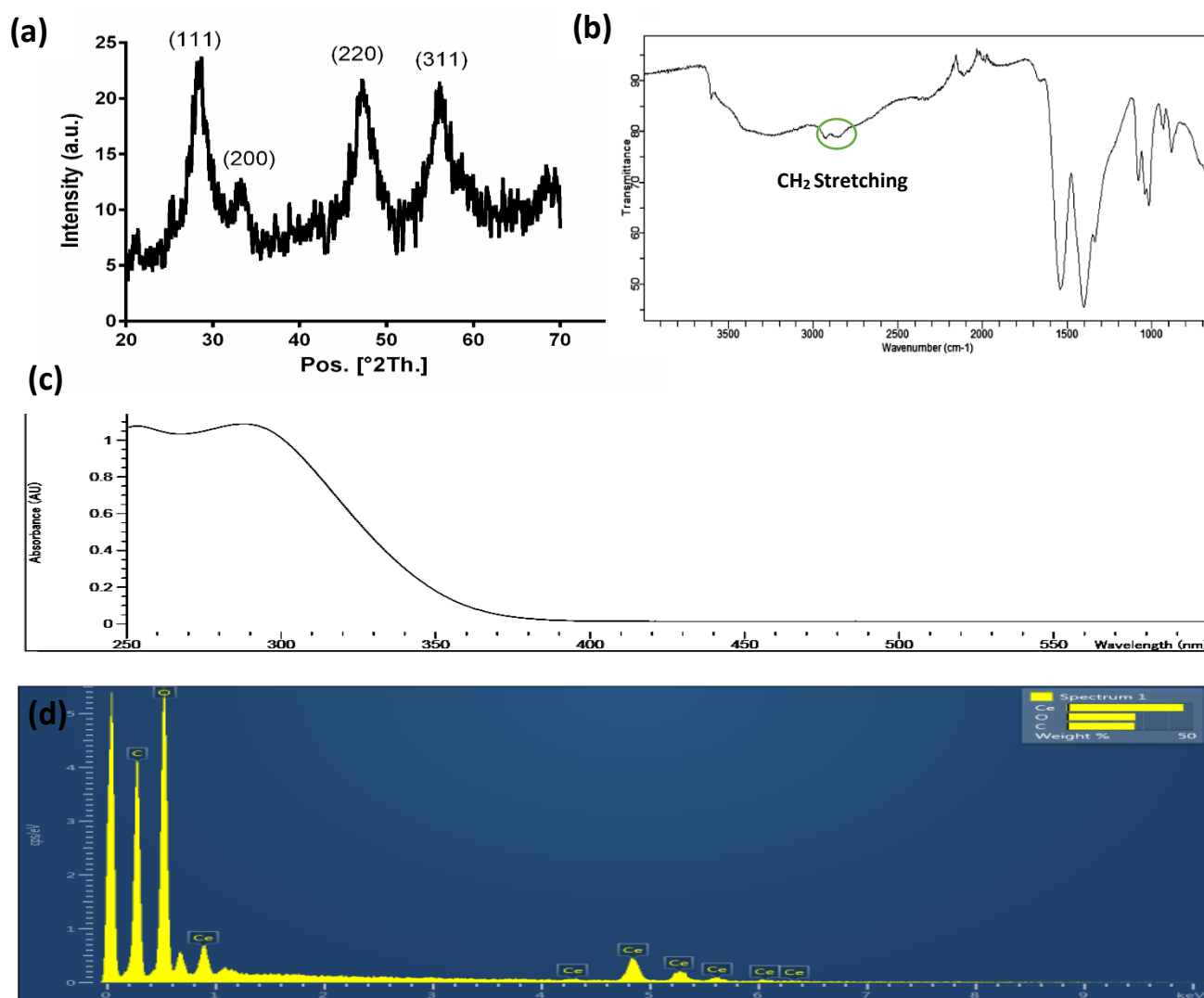


Figure S1. Characterization of ethylene-glycol coated nanoceria (EGCNPs). (a) X-ray diffractogram displaying characteristic planes (annotated) typical of face-centered cubic fluorite structure of nanoceria. (b) FTIR analysis confirming the presence of ethylene glycol (and ethylene glycol acetates) coating as indicated by the appearance of methylene stretching two small peaks in the spectra. (c) UV-Vis spectrum. (d) EDX elemental analysis.

## EuCNPs Characterisation

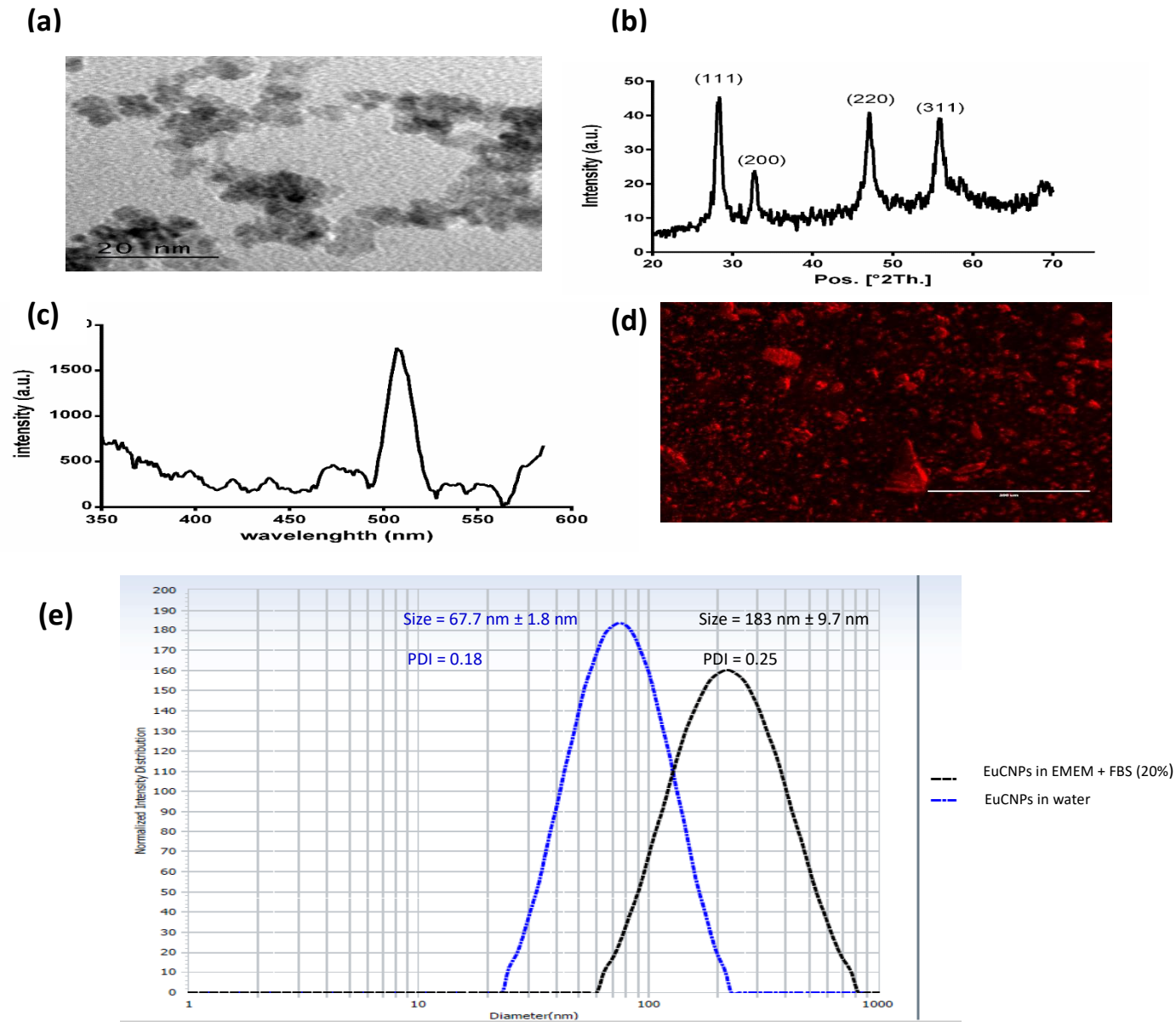


Figure S2. Characterization of europium-doped nanoceria (EuCNP). (a) TEM image showing particles size of 5 - 10 nm. (b) XRD analysis typical of nanoceria fcc crystal, (c) Excitation spectrum of EuCNPs with emission detected at 612 nm, multiple excitation wavelengths can be used to detect the particles at that particular emission wavelength, (d) Fluorescent microscope image of EuCNPs powder showing strong red fluorescence, excitation and emission were detected using RFP-compatible settings (excitation at 531 nm/40 nm, emission at 593 nm/40 nm) using EVOS microscope, (e) DLS data of EuCNPs in both distilled water and EMEM + FBS 20%)

## Mass Spectra of EGCNPs coating

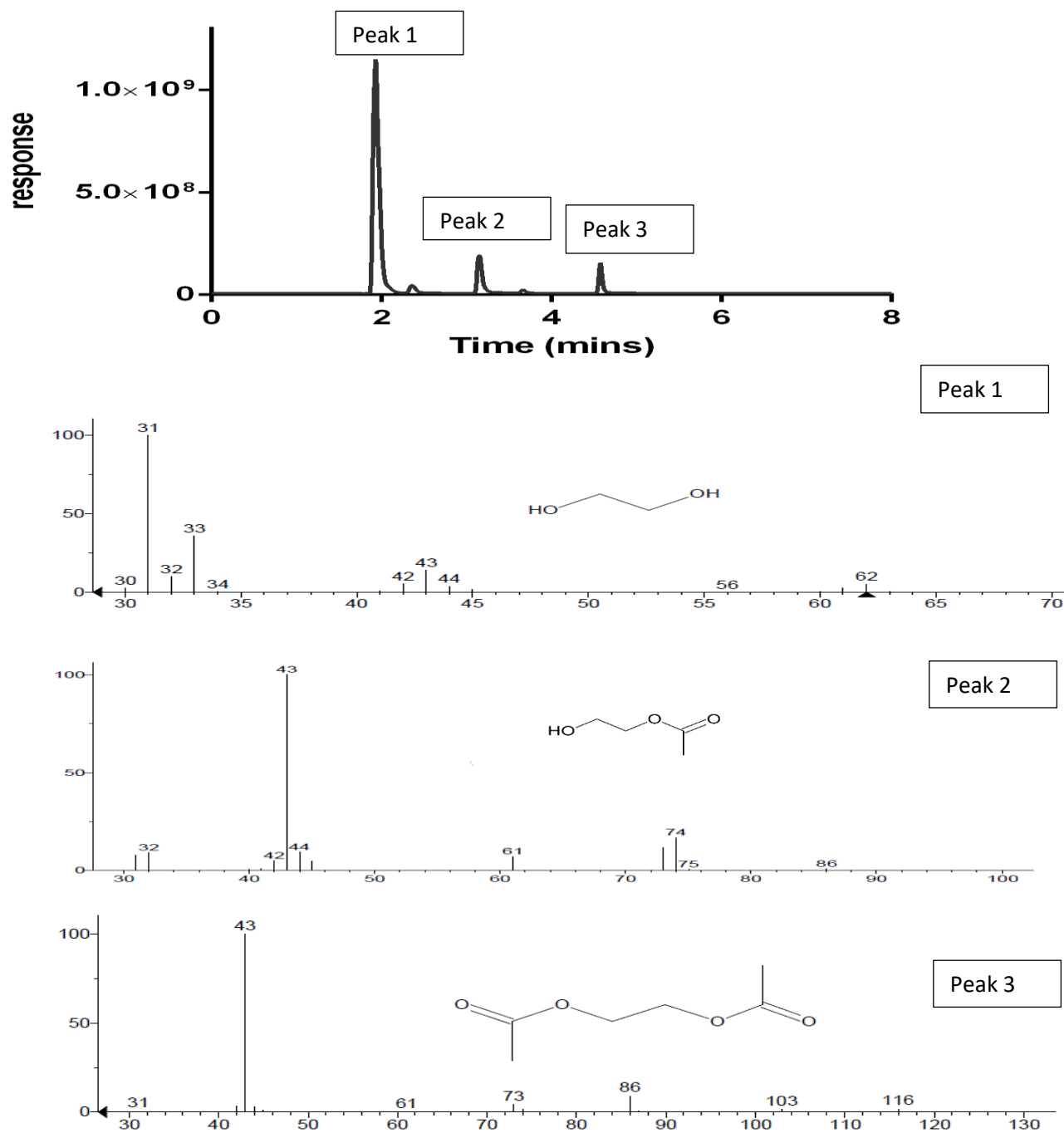


Figure S3. Gas chromatography - mass spectrometry analysis of EGCNPs coating after decomposition in TGA. The chromatogram is showing the three main peaks separated from the decomposed coating and mass spectrum of each peak is displayed confirming the composition to be ethylene glycol, ethylene glycol acetate and ethylene glycol diacetate.

## EDX analysis for uptake and localization

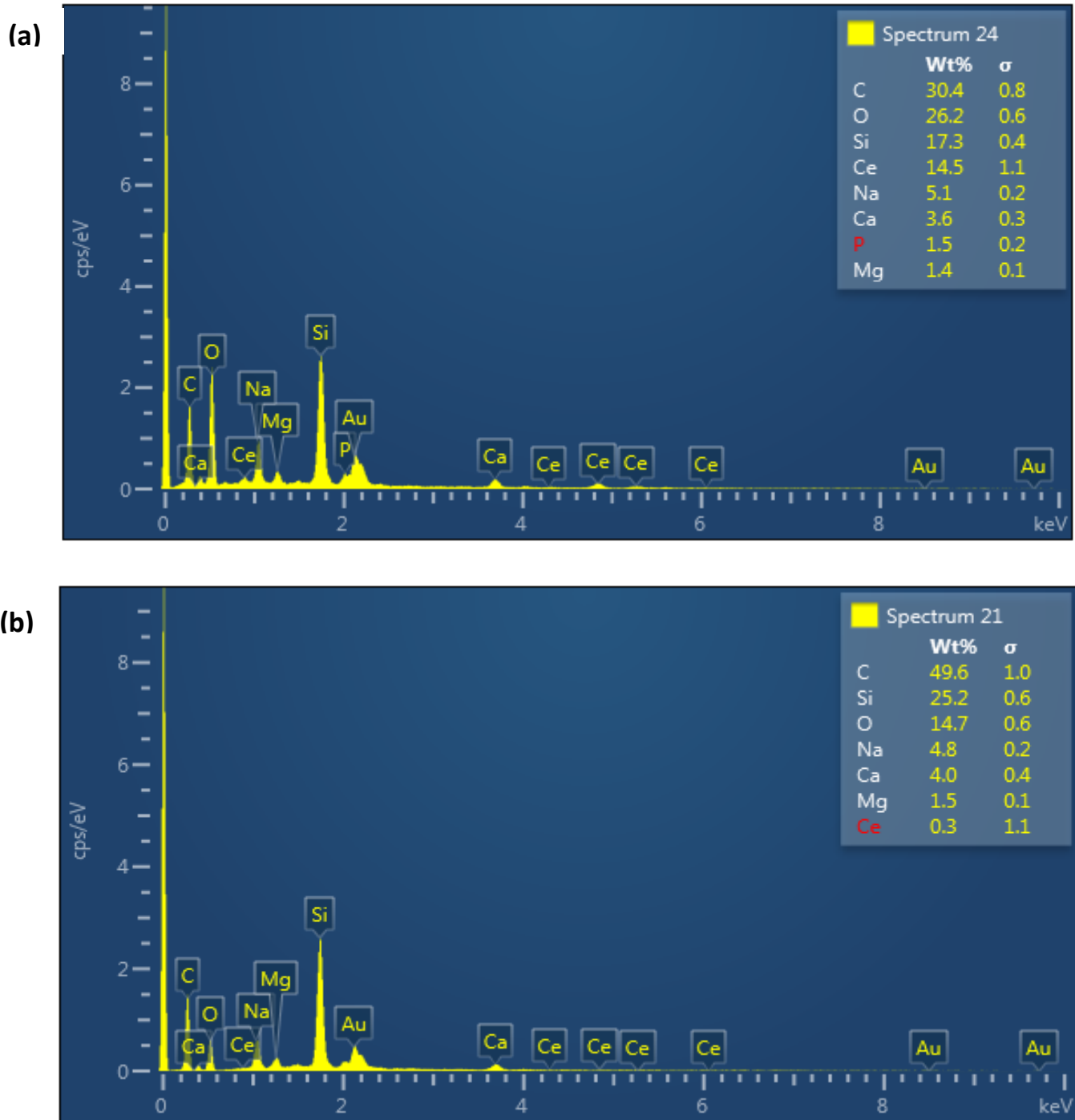
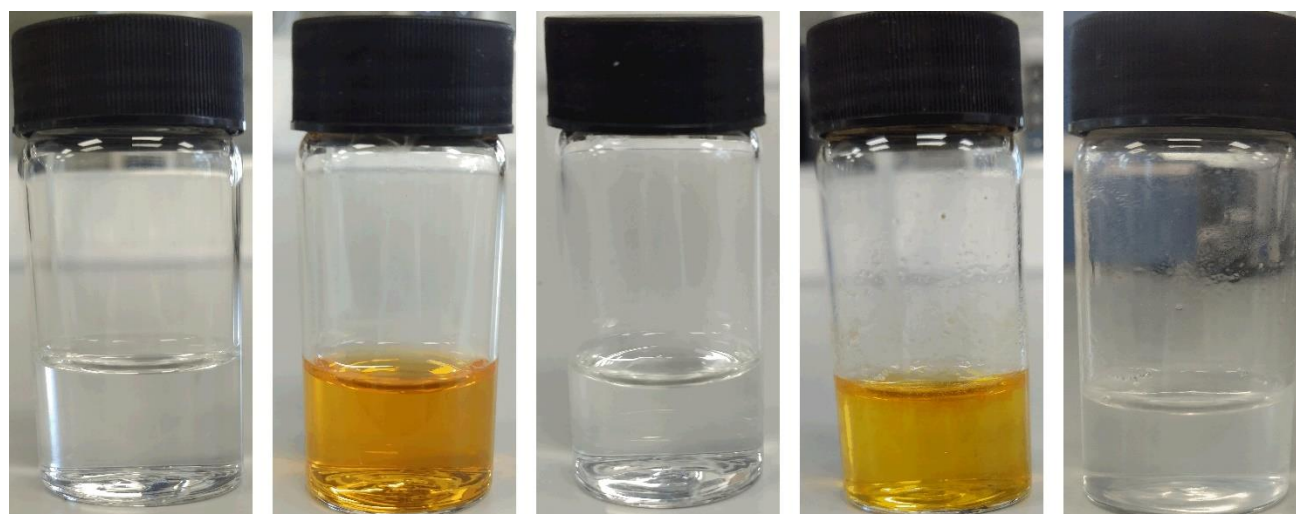


Figure S4. EDX analysis of human lens epithelial cells compartments. (a) a sample spectrum in the cytoplasm (perinuclear region) showing significant uptake of cerium (Ce) that was autodetected. (b) a sample spectrum in the nucleus with no or negligible cerium, this case cerium was not autodetected and was manually input for comparison. Silica (Si) is present in both samples because of mounting on glass cover slips.



EGCNPs (1 mg/ml in water)

After addition of H<sub>2</sub>O<sub>2</sub> (1 drop, 30% w/v)

After boiling for 5 min

Re-addition of H<sub>2</sub>O<sub>2</sub> (1 drop)

Aging for 14 days

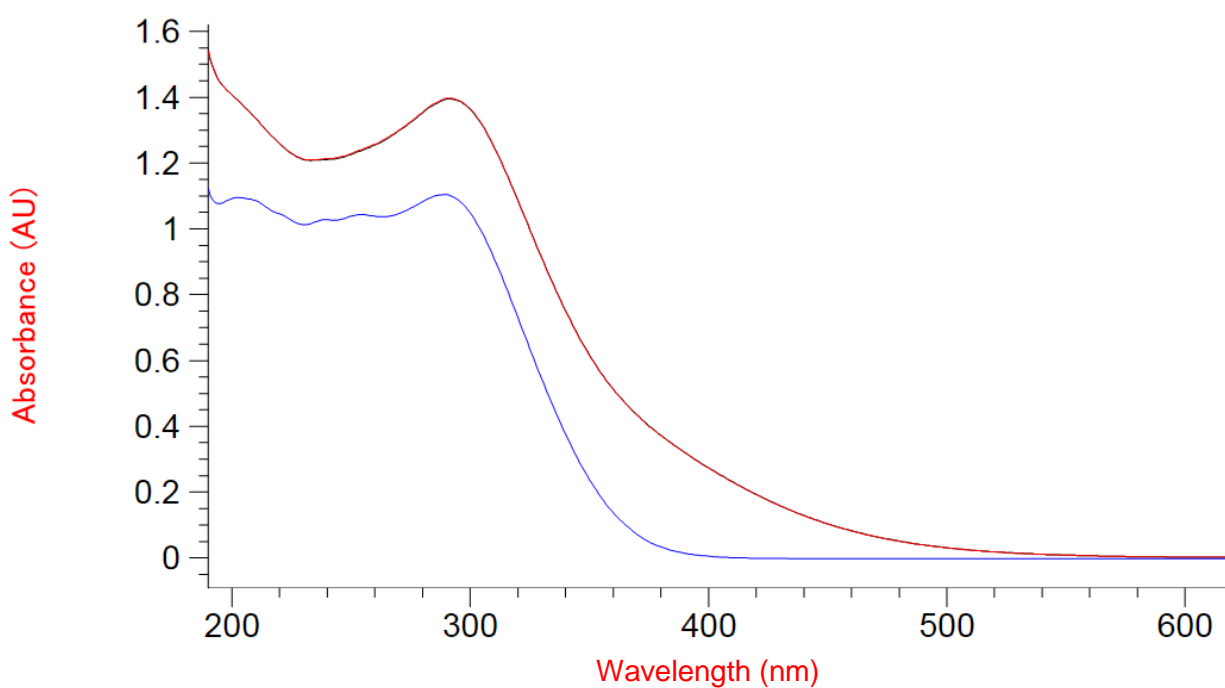


Figure S5. Catalytic and regenerative properties of EGCNPs. Oxidation of Ce<sup>+3</sup> (colourless) into Ce<sup>+4</sup> (orange) upon hydrogen peroxide addition. A red shift in the UV absorption of the resulting orange solution corresponding with this change was observed; colourless Ce<sup>+3</sup> (blue spectrum), Ce<sup>+4</sup> (red spectrum). The reduced colourless solution (Ce<sup>+3</sup>) was retrieved by either boiling or leaving the oxidized solution at ambient conditions (14 days). The reduced solution (Ce<sup>+3</sup>) was reacted again with freshly added hydrogen peroxide turning orange.



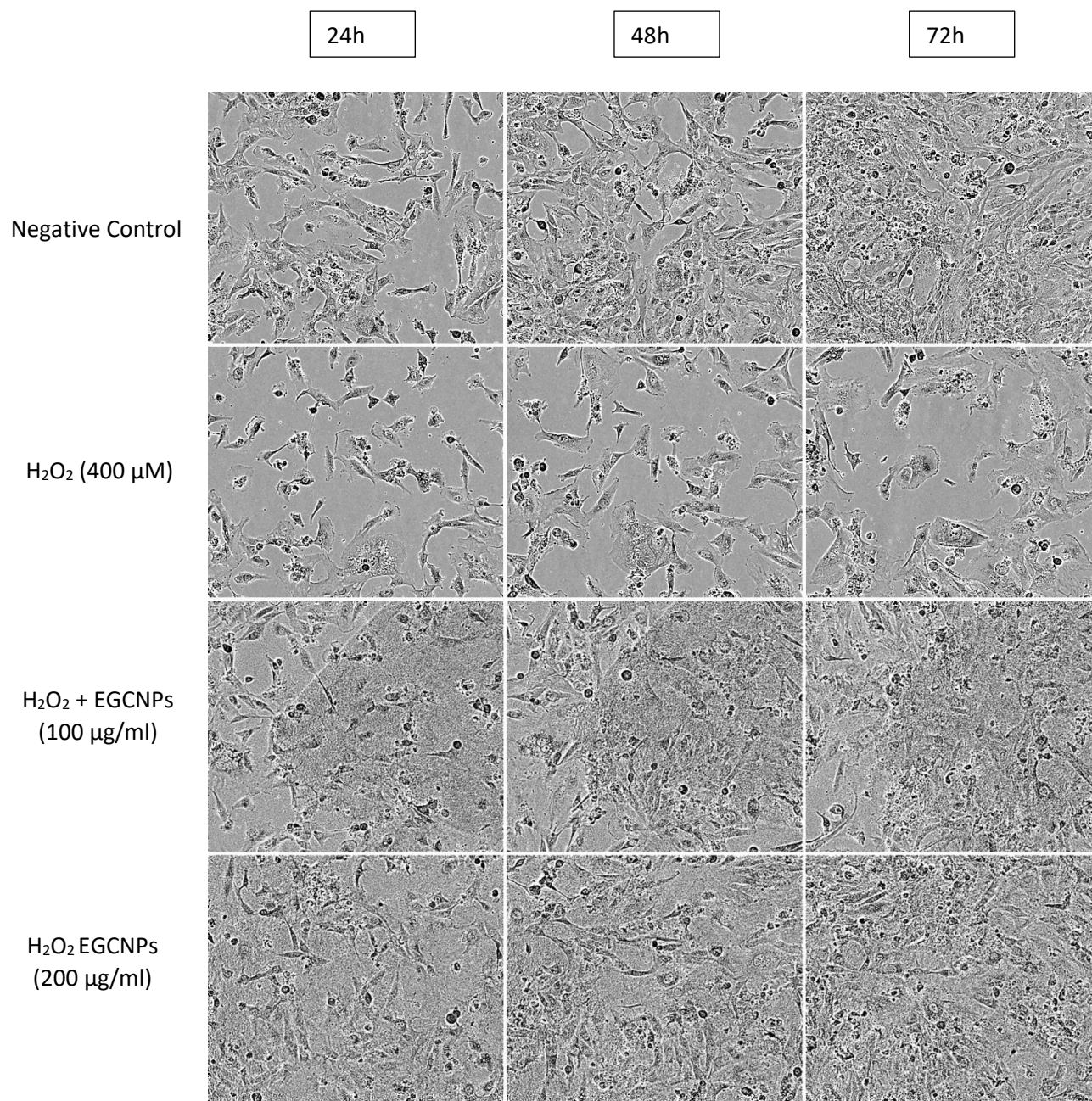


Figure S6. Live cell analysis of human lens epithelial cells showing protective effect of EGCNPs against oxidative stress induced by H<sub>2</sub>O<sub>2</sub>. Cells were incubated with H<sub>2</sub>O<sub>2</sub> with or without treatments and allowed to proliferate for 3 days at 37°C, 5% CO<sub>2</sub>. Images were taken every 2 h using Incucyte S3 system. EGCNPs-treated cells were able to reach full confluency within 3 days while non-treated cells (H<sub>2</sub>O<sub>2</sub> only) proliferation was significantly inhibited by H<sub>2</sub>O<sub>2</sub>.

## Experimental

### Powder X-ray diffraction (XRD)

The crystalline structure of the powder EGCNPs nanoparticles was determined from the diffractograms obtained using an x-ray diffractometer (X'Pert PRO, PANalytical) which operated with Cu  $K\alpha_1$  radiation source ( $\lambda=1.5406$ ) at 30 mA and 40 kV at continuous scanning mode. The scan range operated from 20 - 70 ( $2\theta$ ) with a step size of 0.017 and scan step time of 50.16 seconds. EGCNPs crystallite size ( $d_{XRD}$ ) was calculated from the characteristic diffraction peak (1 1 1) using Scherrer equation:

$$d_{XRD} = \frac{0.94\lambda}{FWHM \cos \theta}$$

where  $\lambda$  is the x-ray radiation wavelength,  $\theta$  is the Bragg diffraction angle for (1 1 1) plane, and FWHM is full width half maximum of the diffraction peak at (1 1 1).

### Transmission electron microscopy (TEM)

TEM was used to determine the morphology and particle size of the nanoparticles. EGCNPs were suspended in distilled water and sonicated for 5 min. 1 drop of the suspension was placed on a holey carbon coated 300 mesh copper grid, left to dry and visualized using TEM operating at 200 kV (Jeol-2010, Tokyo, Japan). The average particle size was determined by measuring the diameter of 100 particles from different spots on the grid.

### SEM-EDX

Scanning electron microscopy equipped with energy dispersive x-ray spectroscopy (SEM-EDX) (Jeol JSM-7100f) was used to identify the elemental composition of nanocerium. Dried EGCNPs were spread on carbon coated aluminium stubs (13 mm) and visualized by SEM-EDX. EDX spectra were acquired and processed using Aztec software (Oxford Instruments).

### UV-Vis Spectroscopy, FTIR and fluorescence measurements

UV-vis absorption spectra of a diluted nanoparticles dispersion (400  $\mu\text{g}/\text{ml}$ ) were obtained using a spectrophotometer (Cary 8454 UV-Vis, Agilent) operating at wavelengths between 200 - 800 nm. Fourier transform infrared spectra were acquired on the dry nanoparticles using FTIR spectrometer (Cary 630 FTIR, Agilent). Emission and excitation spectra were measured in an F-bottom black 96-well plate using a spectrofluorometer (Clariostar, BMG LABTECH)

### Dynamic light scattering and colloidal stability

A stock dispersion of EGCNPs was prepared in distilled water (5 mg/ml) and sonicated for 10 min at room temperature. The dispersion was then diluted to a final concentration of 500  $\mu\text{g}/\text{ml}$  in distilled water or EMEM containing 20% foetal bovine serum. The particle size and

zeta potential measurements were taken using dynamic light scattering (DLS) (Nanoplus, Particulate Systems). Each DLS measurement consisted of 20 accumulations. All measurements were done in triplicates. The colloidal stability of EGCNPs and non-coated nanoceria was measured by allowing nanoparticles dispersions (400 µg/ml) to settle over a week at room temperature. The concentration of the nanoparticles in the supernatant was measured each day spectrophotometrically at 300 nm.

### **Uptake studies of EGCNPs and EuCNPs using confocal imaging**

Human lens epithelial cells were seeded in black 96-well plates with clear bottom surfaces (Corning 353219) and established for 24 h. The cells were then incubated with 200 µg/ml EGCNPs or EuCNPs for 24 h, fixed with paraformaldehyde (4%, 10 min) then permeabilised with Triton-X-100 (0.2%, 10 min). The cells were then counterstained with Hoechst 33342 (Thermofisher) for 10 minutes (2 µg/ml in PBS). Cells were washed with 1X PBS and visualised using a TCS SP5 confocal laser scanning microscope (Leica, Germany). Fluorescent signals from both the nanoparticles and the nuclear stain were collected sequentially at same focal plane using a 20x dry objective (numerical aperture = 0.4) and a pinhole size of 0.9 AU. For EGCNPs detection, cells autofluorescence was first photobleached using high intensity laser power for 2 min, and EGCNPs were detected at (530-560 nm) using 488 nm excitation laser line. In case of EuCNPs, the signal was strong enough compared to cellular autofluorescence and the nanoparticles were directly detected at 612 nm using 514 nm excitation laser line.