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Product Information

Sunstone® Upconverting Nanocrystals UCP 545, Carboxylated

Catalog Number **61334**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Sunstone® Upconverting Nanocrystals (UCP) are a novel and proprietary class of rare earth doped nanoparticles of small size, high quantum efficiency, and high photoluminescent intensity that have been functionalized for use in industrial and life sciences applications. UCP are synthesized using specific compositions of individual rare earths and other host elements ($NaYF_4$). Ytterbium serves as the element that initially absorbs the electromagnetic radiation, while other rare earths, such as erbium, holmium and thulium, serve as the emitting elements at the center of the crystal.

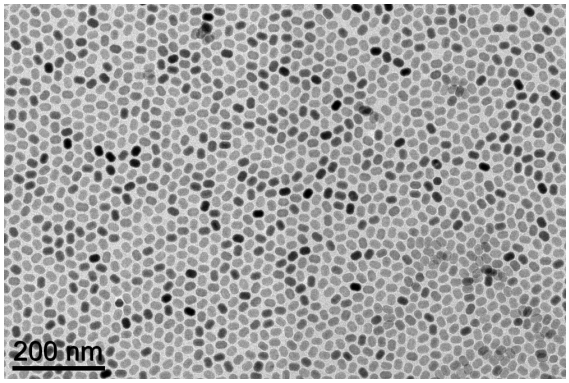
Upconversion luminescence is based on the absorption of two or more low-energy (longer wavelength, typically infrared) photons by a nanophosphor crystal followed by the emission of a single higher-energy (shorter wavelength) photon. This is a unique process and does not occur in nature.

Upconverting materials have been used in a broad variety of life science applications, including:[1,2]

- Immunohistochemistry
- Immunocytochemistry
- Multiplex immunoassays
- Nucleic acid microarrays
- *In vivo*, *in situ*, and *ex situ* biomedical imaging
- Flow cytometry
- Enzymatic assays
- Fluorescence resonance energy transfer (FRET) bioanalytical assays

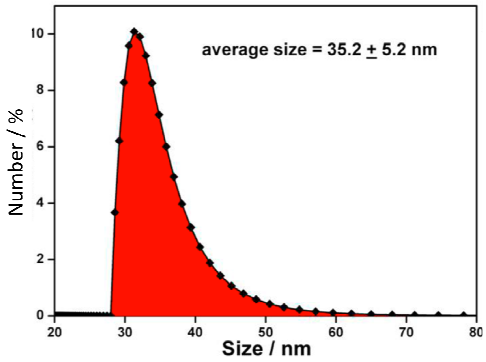
Properties of Sunstone Upconverting Nanocrystals UCP 545, Carboxylated:
Physical form: Lyophilized powder
Excitation maximum: 976 nm
Emission maxima: 545 nm, 660 nm
Diameter: 40±15 nm
Functionality: Carboxylate (terminal COOH groups)
Crystal Host: Sodium Yttrium Fluoride ($NaYF_4$)
Activators: Ytterbium (Yb) and Erbium (Er)
Crystal Formula: $NaYF_4$, Yb, Er
Stabilizer: PEG

Figure 1.
Morphology: Rods



Transmission electron microscope (TEM)

Figure 2.
Crystal Size: ≤40 nm



Dynamic Light Scattering (DLS)

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Nanocrystals can be suspended directly in water at desired concentration for conjugation. Prior to use sonicate the stored stock suspensions to disperse the nanocrystals.

Storage/Stability
Store the product at 2–8 °C.

After preparation, store stock suspensions for 4–6 weeks at 2–8 °C. Do not freeze suspensions of nanocrystals

Procedures
A. Antibody Complex Formation
Materials and reagents required for antibody complex formation:

- Antibody Solution (100 µg/0.1 mL)
- 10 mM HEPES buffer, pH 7.2,
- HBS Buffer – 10 mM HEPES with 154 mM NaCl, pH 7.2
- Glass centrifuge tubes capable of withstanding 1,000 × *g*
- Centrifuge capable of providing 614 × *g*
- Vortex mixer or rotary shaker
- Glass Pasteur pipettes
- Carboxylated Sunstone Upconversion Nanocrystals as a suspension in water, ~10 mg/mL
- Fetal Bovine Serum (FBS), Catalog No. F2442 and bovine serum albumin (BSA), Catalog No. A7979

1. Nanocrystal Preparation
   a. Sonicate the suspension of Carboxylated Nanocrystals (~10 mg/mL) for 20 seconds. After sonication, transfer 50 µL of suspension into a glass centrifuge tube. Add 1 mL of HBS Buffer to the tube and centrifuge the mixture at 1,000 × *g* for 15 minutes. Discard supernatant.
   b. Resuspend the particles in 3 mL of 10 mM HEPES buffer, pH 7.2. Sonicate the suspension for 20 seconds, then centrifuge at 1,000 × *g* for 15 minutes and discard supernatant.
   c. Repeat step 1b once.

2. Antibody-Nanocrystal Complex Formation
   a. In a separate tube, prepare the Antibody-HBS Solution by adding 20 µL of antibody to 8 mL of HBS Buffer.
   b. Add 3 mL of the Antibody-HBS Solution to the pelleted particles from step 1c. Sonicate the mixture for 20 seconds. Add the remaining 5 mL of the antibody solution. Mix the resulting suspension on a rotary mixer for 2 hours.

3. Antibody-Nanocrystal Complex Clean-up
   a. After the Antibody-Nanocrystal Complex is formed, centrifuge the suspension at 1,000 × *g* for 15 minutes. Discard supernatant.
   b. Wash the Antibody-Nanocrystal Complex by adding 7 mL of fresh HBS to the pellet. Do not resuspend. Centrifuge the tube at 1,000 × *g* for 15 minutes. Discard supernatant.
   c. Repeat step 3b twice to wash pellet.
   d. Add 3 mL of HBS to the tube. Resuspend the Antibody-Nanocrystal Complex by sonicating for 20 seconds. Add an additional 5 mL of HBS and centrifuge the suspension at 1,000 × *g* for 15 minutes. Discard supernatant.
   e. Repeat step 3d twice.
   f. Add 3 mL of HBS supplemented with 7.5% FBS and 2.5% BSA to the pellet. Resuspend the Antibody-Nanocrystal Complex by sonicating for 20 seconds. Add an additional 5 mL of HBS supplemented with 7.5% FBS and 2.5% BSA and centrifuge the suspension at 1,000 × *g* for 15 minutes. Discard supernatant.
   g. Resuspend the Antibody-Nanocrystal Complex in 2 mL of HBS supplemented with 7.5% FBS and 2.5% BSA.
   h. Store the Antibody-Nanocrystal Complex at 2–8 °C protected from light.

B. Streptavidin Conjugation to Carboxylated Nanocrystals

Materials and reagents required for conjugation of streptavidin to carboxylated nanocrystals:

- *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), Catalog No. E1769
- *N*-Hydroxysulfosuccinimide sodium salt (Sulfo-NHS), Catalog No. 130672
- MES, Catalog No. M5287, to prepare 0.1 M MES buffer, pH 6.0
- Phosphate buffered saline (PBS), pH 7.2
- L-Lysine, Catalog No. L5501
- Glass centrifuge tubes capable of withstanding 5,000 × *g*
- Centrifuge capable of providing 3,000 × *g*
- Magnetic stir plate
- Glass Pasteur pipettes
- Carboxylated Nanocrystals as a suspension in water, ~10 mg/mL
- Streptavidin from *Streptomyces avidinii*, Catalog No. S4762

1. Activation of Carboxylated Nanocrystals
   a. Dissolve 1 mg of EDC and 4 mg of Sulfo-NHS in 4 mL of 0.1 M MES buffer, pH 6.0, containing 1 mg of the activated carboxyl nanocrystals.
   b. Stir the mixture for 8 hours at room temperature.
   c. Centrifuge the mixture at 3,000 × *g* for 6 minutes. Discard supernatent and wash with 4–6 mL of water.
   d. Repeat step 1c.

2. Conjugation Reaction
   a. Resuspend the activated carboxyl nanocrystals in 5 mL of PBS buffer, pH 7.2, containing 0.5 mg of streptavidin.
   b. Incubate suspension at 2–8 °C for 48 hours.
   c. Add 4 mg of L-lysine to the suspension to neutralize any unreacted Sulfo-NHS.
   d. Pellet the streptavidin-nanocrystal conjugate by centrifuging the suspension at 3,000 × *g* for 6 minutes. Discard supernatant and wash the pellet with 4–6 mL of water.
   e. Repeat step 2d twice.
   f. Store suspensions of streptavidin-nanocrystal conjugates at 2–8 °C. Do not freeze.

Note: As a general guideline in existing procedures using streptavidin-FITC, the suggested starting concentration for the streptavidin-nanocrystal is 50% of the strepavidin-FITC concentration. Optimization of the application will be necessary.

Results

Instrumentation Recommendations

The following are representative of instruments suitable for near-IR upconversion phosphorescence using Sunstone Upconversion Nanocrystals, but is not meant to be an all-inclusive list of instruments.

Spectrometers

- Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, USA) with a standard R928 red-sensitive photomultiplier (Hamamatsu Photonics, Japan) was equipped with IR laser diode module C2021-F1 (Roithner Lasertechnik, Austria). An IR laser diode module and a long-pass filter glass RG-850 (Andover Corporation, USA) was mounted to a cuvette holder of the spectrophotometer. Emitted light was collected using bio/chemiluminescence mode of the spectrophotometer from 350–850 nm.[3]

- Fiber-optically coupled USB4000 fluorescence spectrometer (Ocean Optics, USA) using an external continuous-wave laser centered at ~980 nm as the excitation source (Dragon Lasers, China).[4]

Benchtop Scanner

- 96-well FluoroCount multiwell plate reader (Perkin Elmer, USA) modified with an external 980 nm 1.2 W IR laser (Oclaro, USA).[2]

Microscopes

- Inverted fluorescence microscope (Leica Microsystems, Germany) equipped with a 980 nm NIR laser and a Nikon digital camara.[5]
- Epifluorescence microscope (Leica Microsystems, Germany) modified with a 980 nm light from a xenon XBO 75 W lamp.[2]
- Olympus microscopes using 975 diode laser (QPhotonics LLC, USA); with a laser diode driver; Thorlabs LDC 30 65 – 488. Detection: xy translation monitored filter coupled; Ocean Optics, USB 4000

In vivo Imaging

- *In vivo* spectral imaging system (CRI Inc., USA) equipped with a 980 nm diode laser excitation source (B&W TEK Inc., USA).[6]

Other Possible Excitation Laser Sources

- JDSU 3000 series 660 mW Fiber Bragg grating stabilized 976±1 nm pump module (PN 30-7602-660).
- Edmund Optics Fiber Laser 976 nm 450 mW (PN NT62-688)
- Newport LD Module, 980 nm, 220 mW, CW – (Model: LQC980-220E)

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