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New World of Nano-Bio Technology

NEO-LIVE™

Innovative in vivo imaging probe

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Nano Bio Frontier

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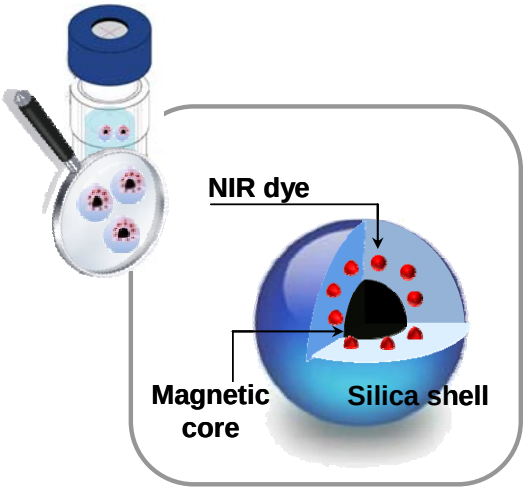
Introduction

NEO-LIVE™ is the multimodal probe for *in vivo* live imaging of both magnetic resonance (MR) and fluorescence imaging. It is a core-shell structure (magnetic NP-silica), and a Near Infra-Red (NIR) fluorescent dye is incorporated in a silica shell (Scheme 1).

In particular, this probe is specially designed for improving the photo-stability and controlling the fluorescent signal intensity. It can greatly be powerful *in vivo* study and detected by some equipment such as Maestro™ (CRI) or IVIS (Xenogen).

Structure design



Scheme 1. Structure of biocompatible NEO-LIVE™. NEO-LIVE™ is a magnetic silica nanoparticle (core-shell), which contains Near-IR fluorescent dyes;
(1) NIR 797 (E_x/E_m = 797/830 nm)
(2) NIR 730 (E_x/E_m = 680/755 nm)
(3) NIR 675 (E_x/E_m = 675/700 nm)

※ Particle size of NEO-LIVE™ is controlled as 50 nm (± 10 %).

Applications

- *In vivo* live imaging without sacrifice
- Deep tissue *in vivo* live imaging
- Long term *in vivo* cell tracking
- Conjugation with various biomolecules

Performance Tests

In vivo Imaging

Demonstrations of *in vivo* imaging with NEO-LIVE™.

Figure 1-6 are *in vivo* live images tested in brain, liver, spinal code, articular capsule, and sentinel lymph node of mouse. An injected site could be successfully detected without a sacrifice.

(※ Note : NEO-LIVE™ Magnoxide797 was used in this tests.)

Sensitivity (Detection limit)

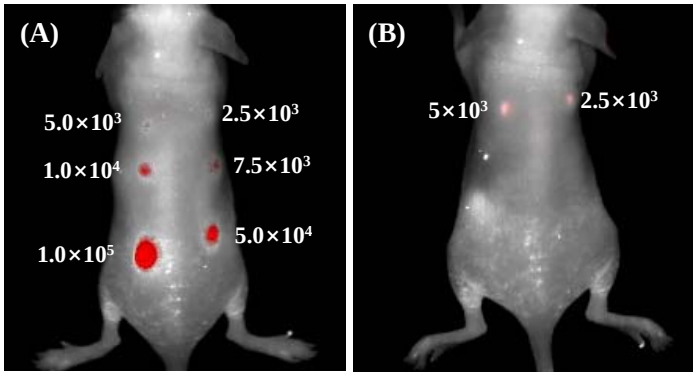
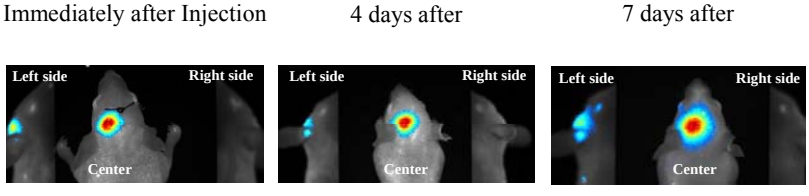


Figure 1. High sensitivity of NEO-LIVE™ (Detection limit test). Small number of A549 cells labeled with NEO-LIVE™ (Magnoxide 797) was injected (A) subcutaneously for testing the in vivo imaging detection limits. We were able to observe labeled cells with up to a minimal numbers of 2.5×10³. (B) Tried again under same condition of small numbers of injection.

Brain (Mouse)



Average Intensity : 0.00111
Area : 91.83mm²

Average Intensity : 0.000879
Area : 95.21mm²

Average Intensity : 0.000279
Area :148mm²

Figure 2. *In vivo* images of mouse brain injected with U87MG cancer cells labeled by NEO-LIVE™ (Magnoxide797). 5×10⁵ U87MG cancer cells were injected into the brain after labeling with NEO-LIVE™ (Magnoxide797) for imaging the brain cancer. Fluorescence signal detected in vivo of NEO-LIVE™ was so high that it was enough to penetrate skulls. And NEO-LIVE™ was so photostable that we expect to be useful to long term *in vivo* study.

Mouse model

Spinal code (Mouse)

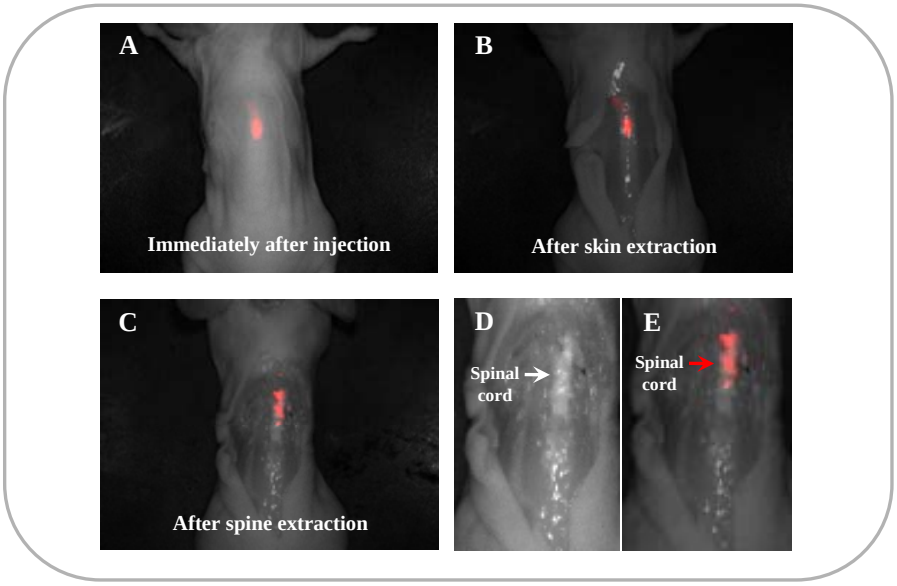


Figure 3. *In vivo* images of mouse spinal code by NEO-LIVE™ (Magnoxide797). 1 × 10^5 A549 cells labeled by NEO-LIVE™ were injected into the spinal cord though abdominal surgery, and then we detected the fluorescence signal in vivo. (A) immediately after cell injection, (B) after skin extraction, (C) after spine extraction; to confirm the injected cell location in spinal cord, we remove a muscle and spine. It was verified that injected cell was located in the spinal cord and fluorescence signal of injected cell was enough to penetrate spine. (D) Enlarged image of spinal cord (white light), and (E) Enlarged fluorescence / white light image of spinal cord after merging.

NEO-LIVE™ is designed for tracking a few labeled cells in deep tissue. The encapsulation of NIR dyes in a silica significantly enhances its quantum yield.

Liver (Mouse)

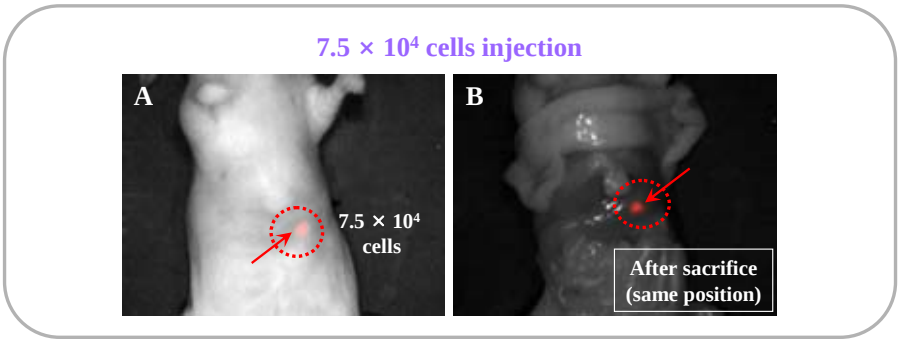


Figure 4. *In vivo* image of mouse liver by NEO-LIVE™ (Magnoxide797). (A) Once 7.5 × 10^4 A549 cells labeled with NEO-LIVE™ were injected into the liver of BALB/C nude mouse, the image was taken using Maestro™ In Vivo Imaging System. (B) Image of same position after sacrifice.

Mouse model

NEO-LIVE™ is remarkably powerful for a long-term *in vivo* live imaging due to photochemical stability resulting in minimal photo-bleaching even long exposure time under the UV.

Articular capsule (Mouse)

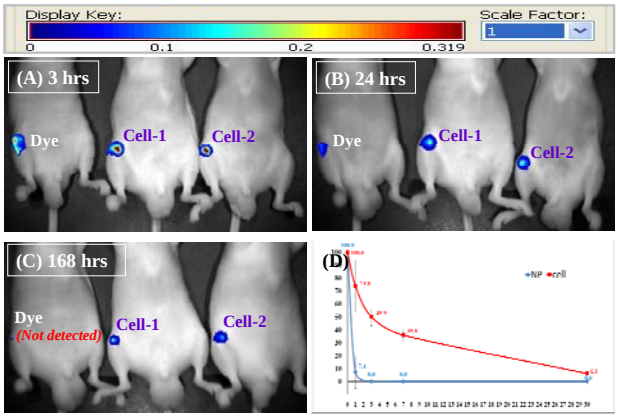
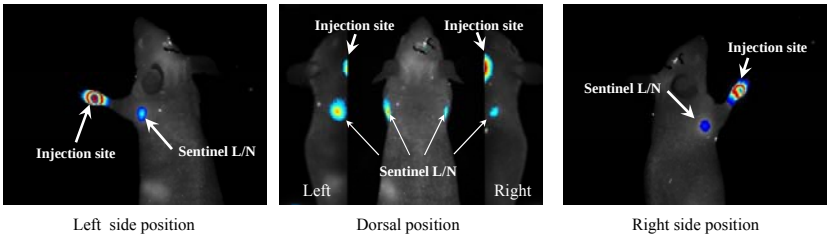


Figure 5. *In vivo* images of a mouse articular capsule by NEO-LIVE™ (Magnoxide797) and signal degradation graph. (A - C)1 × 10^6 chondrocyte labeled by NEO-LIVE™ (Magnoxide797) was injected into the articular capsule, and then we detected. (D) Increasing the time, fluorescence signal from labeled cells was slowly decreased while that of dye itself was rapidly decreased. After 7 days (168 hrs), fluorescence signal of NEO-LIVE™ was about 30 % and that was 7 % even after 30 days.

Sentinel lymph node (Mouse)



Left side position | Dorsal position | Right side position

Figure 6. *In vivo* lymphatic drainage imaging of a mouse injected with NEO-LIVE™ (Magnoxide797). NEO-LIVE™ 150 ㎍ (25 ㎕, 6 ㎎/㎖) and 90 ㎍ (15 ㎕, 6 ㎎/㎖) was injected intracutaneously into the left (150 ㎍) and right (90 ㎍) sides on the basis of the middle digit of upper extremity. The axillary lymph node was clearly imaged through the skin.

Rat model

NEO-LIVE™ Magnoxide797

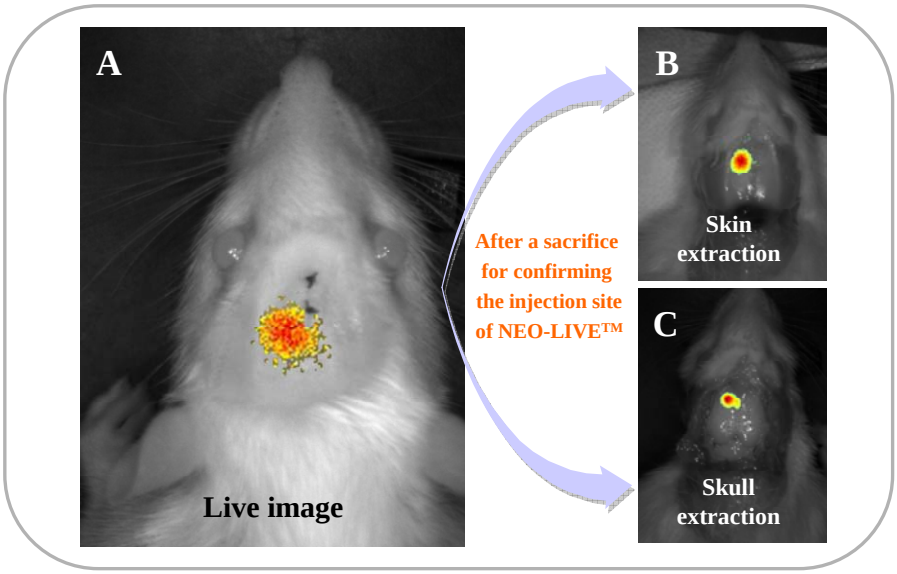


Figure 7. *In vivo* images of a rat brain injected with NEO-LIVE™ (Magnoxide797). Once in vivo live imaging was done, the injection site was sacrificed for clarifying the performance by NEO-LIVE™ (Magnoxide797).

NEO-LIVE™ Magnoxide675

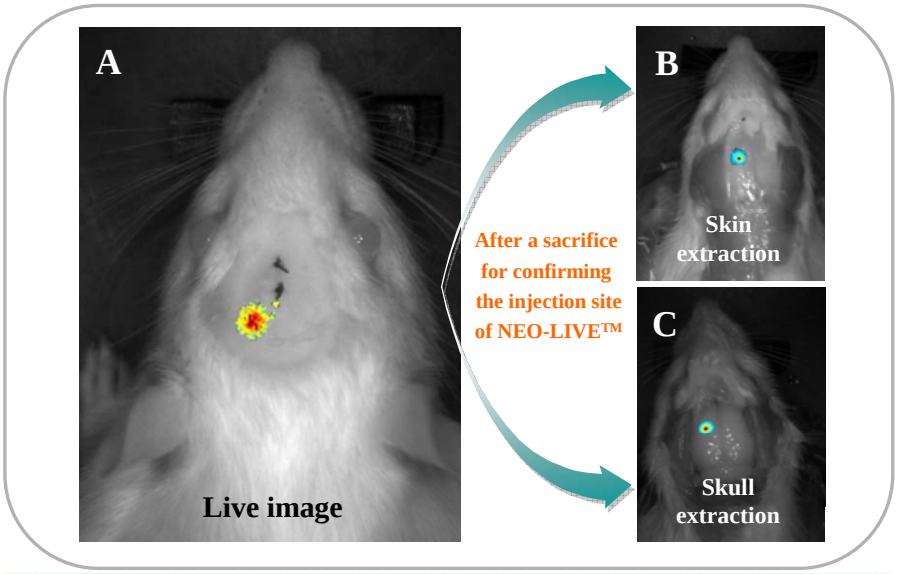


Figure 8. *In vivo* images of a rat brain injected with NEO-LIVE™ (Magnoxide675). Once in vivo live imaging was done, the injection site was sacrificed for clarifying the performance by NEO-LIVE™ (Magnoxide797).

Rat model

Articular capsule

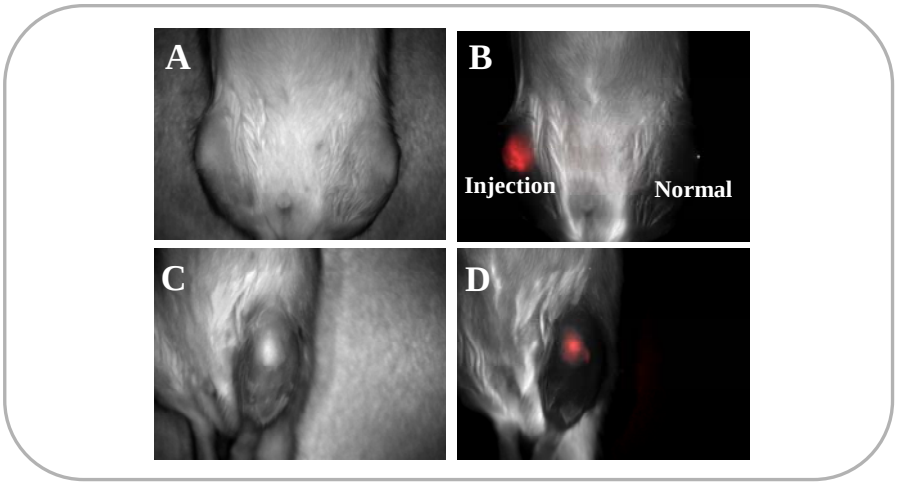


Figure 9. (A-B): *In vivo* image of articular capsule of rat (after injecting 5×10⁵ chondrocyte labeled by NEO-LIVE™ (Magnoxide 797)). (A) white light image and (B) fluorescence image. **(C-D) articular capsule in vivo image (after injecting 1×10⁵ chondrocyte labeled by NEO-LIVE™ (Magnoxide 797))** (C) white light image and (D) fluorescence image

Advanced feasibility

Conjugation with biomolecules

By modifying (Amine or Carboxyl) a surface of **NEO-LIVE™**, you can conjugate with various biomolecules just like protein, antibody, drug, peptide, and DNA. Magnoxide (PEG) is designed for enhancing biocompatibility *in vivo* vascular and lymphatic imaging.

In addition, you can use NEO-LIVE™ in a number of biomedical applications such as targeting, bio-imaging, cell sorting, drug delivery and therapy.

Products

Table 1. Contents and Storage information

Material	Wavelength	Concentration	Storage
NEO-LIVE™ Magnoxide730	E_x/E_m = 680/755 nm	2 mg /ml (25.1 nM) in borate buffer,	2-6℃ **Do not freeze or dry**
NEO-LIVE™ Magnoxide797	E_x/E_m = 797/830 nm		
NEO-LIVE™ Magnoxide675	E_x/E_m = 675/700 nm		

Products List - Prices information is inquired from our customer Service Department.

Cat. No.	Product Name	Wavelength λ_{max} (E_x /E_m)	Surface chemical reactor
M7985-01	Magnoxide730	680 / 755 nm	Hydroxyl group (-OH)
M7985-03	Magnoxide730 (Amine)	680 / 755 nm	Amine group (-NH_3)
M7985-04	Magnoxide730 (Carboxyl)	680 / 755 nm	Carboxyl group (-COOH)
M7985-05	Magnoxide730 (PEG)	680 / 755 nm	PEG
M7983-01	Magnoxide797	797 / 830 nm	Hydroxyl group (-OH)
M7983-03	Magnoxide797 (Amine)	797 / 830 nm	Amine group (-NH_3)
M7983-04	Magnoxide797 (Carboxyl)	797 / 830 nm	Carboxyl group (-COOH)
M7983-05	Magnoxide797 (PEG	797 / 830 nm	PEG
M7675-01	Magnoxide675	675 / 700 nm	Hydroxyl group (-OH)
M7675-03	Magnoxide675 (Amine)	675 / 700 nm	Amine group (-NH_3)
M7675-04	Magnoxide675 (Carboxyl)	675 / 700 nm	Carboxyl group (-COOH)
M7675-05	Magnoxide675 (PEG)	675 / 700 nm	PEG

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Further information on Biterials products is inquired from your local distributor or Biterials directly.

Biterials products are high-quality materials intended for research purposes mainly. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet (MSDS) provided for each product, other regulatory considerations may apply.

Korean distributor
Woongbee MeDiTech Inc.
RM 1309-10 Biz Center,
SK Techno Park 190-1,
Sangdaewon-1 dong, Jungwon-ku,
Seongnam, Kyunggi-do 462-807,
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Tel : +82-31-776-3300
Fax: +82-31-776-3303

Japanese distributor
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9-7, Hongo 2-chome,
Bunkyo-ku, Tokyo 113-0033, Japan.
Tel : +81-3-5684-1620
Fax: +81-3-5684-1775

Appendix I. *Protocol for Experiments*

Before you begin

• Materials required but not provided
- Cell culture media, Ultrasonic bath, Centrifuge machine

Preparation of Labeling Solutions

1.1 Put NEO-STEM™/LIVE™ into a new tube.
(**Note: Adjust the final concentration of NEO-STEM™/LIVE™ at/around 0.2 ㎎/㎖. The concentration for MSCs is 0.4 ㎎/㎖. Most culture media can be used.*)

1.2 Centrifuge at/above 12,700 ×g (12,000 rpm) for 10 min.

1.3 Remove the supernatant completely from a tube.

1.4 By adding growth media into a tube, make a concentration of 0.2 or 0.4 ㎎/㎖.

1.5 To disperse, sufficiently sonicate it for 5 min (in an ultrasonic bath).
(**Note: Please DO sonicate sufficiently!! The uptake efficiency strongly depends on the dispersibility of NEO-STEM™/LIVE™. Ultrasonic condition : above 40 kHz and 300 W.*)

Cell Labeling (*In-vitro* study)

2.1 Prepare the cell in a culture dish or flask.
(**Note: Adherent cells were seed in plates and allowed to grow for some time to adhere.*)

2.2 Remove media from a culture dish or flask.

2.3 Add NEO-STEM™/LIVE™ labeling solution (*of Process-1*) to cells.

2.3 Incubate the cell for 2~24 hrs to label cells.
Ex) NEO-STEM™ *: A549 (2~3 hrs), HeLa (4~6 hrs), hMSCs (above O/N).*
NEO-LIVE™ *: Above 24 hrs*
(**Note: The cell labeling time of surface modified products can be different.*)

2.4 Wash cells 3 times with a complete growth medium or PBS.

2.5 Visualize labeled cells using any suitable fluorescence microscopy, flow cytometry or the imaging system with appropriate filters.

Histological observation

3.1 NEO-STEM™/LIVE™ is so stable in organic solvents (alcohol, xylene) that you can make paraffin section or frozen section.

Live Imaging (*In-vivo* study)

4.1 For only NEO-LIVE™ users.
4.1.1 *In-vivo* fluorescence live-image (Near Infrared; NIR).
(**Note: By using in vivo imaging system such as Maestro™ and IVIS, etc.*)

4.2 For NEO-STEM™/LIVE™ users.
4.2.1 MR image.
(**Note: NEO-STEM™/LIVE™ is T2-weighted MRI agents; induces very hypointense and dark signal of the proton in H_2O.*)

※ This protocol is provided for users who study on in-vitro /vivo images using NEO-STEM™ or NEO-LIVE™ of Biterials.