Cell tracking products:
LumiLys 780, MultiLys 780 T_2, MultiLys 625 T_2 and MultiLys 650 T_1

1. Product description

1.1 Introduction

To understand phenomena involved in a pathological process, it is often useful to monitor the movements of living targeted cells and to locate them with non-cytotoxic specialized probes. Our cell tracking products are fluorescent nanoparticles with an emitting wavelength range fitting with lasers and filters for both *in vitro* and *in vivo* optical imaging devices.

LumiLys 780 nanoparticles are specifically designed for *in vitro* labeling of various cell types (cancer, strain, immune) before their *in vivo* injection / transplantation and allow monitoring for several weeks. Indeed, the encapsulation of several hundred of fluorescent molecules (Cy7) within a mineral silica shell gives to the nano-objects an exceptional brilliance and resistance to metabolic and photo-chemical degradations (photobleaching).

MultiLys 780 T_2 and MultiLys 625 T_2 products are designed for multimodal detection by MRI and computed tomography (CT) in addition to luminescence imaging. Magnetic resonance imaging (MRI) is an efficient technique for cell tracking applications due to its very good resolution of soft tissue. The X-ray tomography also has a good resolving power and allows easy coupling of functional images with anatomical images. Finally, the fluorescence properties of our multimodal nano-probes will enable you to visualize (by microscopy or using *in vivo* imager) and quantify (flow cytometry) easily the cell labelling intensity.

Our multi-modal nanoprobes **MultiLys 625 T_2, MultiLys 780 T_2** are optimized to label a large number of cell lines by endocytosis. After being labeled *in vitro* and then injected *in vivo*, cells can be monitored using fluorescence, MRI or X-ray absorption properties.

MultiLys 650 T1 is a positive contrast agent by MRI (T1) which is also fluorescent. However contrast given using CT is only very week.

1.2 Product format and storage

Cell tracking products are supplied as dried powders. Suspension (1 mg/mL for instance) has to be reconstituted by mixing the dried powder and the furnished medium (5% glucose-water 5% solution), then sonicated few minutes in an ultrasonic bath[1].

Dried nanoparticles can be preserved indefinitely in the absence of light.

Nanoparticles in suspension should be used within 7 days and sonicated just before using to ensure a good dispersion.

Cell tracking products contain no preservatives. Avoid any microbial contamination during use.

1.3 Quality control

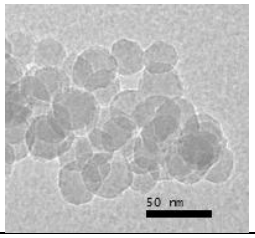
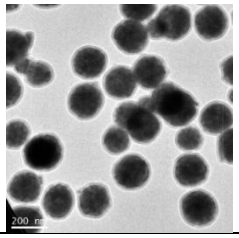
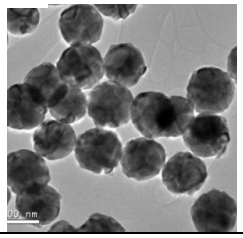
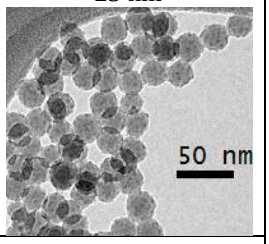
Cell tracking products are tested to ensure lot-to-lot consistency. Size of the nanoparticles is examined by Transmission Electron Microscopy. Fluorescence quality is controlled by spectrophotometry.

1.4 Security

For laboratory and animal research use only. Not for human or animal therapeutic or diagnostic use. Make sure to carefully observe the legislation on animal experimentation.

[1] You can use very common ultrasonic bath usually used for vessels, mechanical pieces or jewelry cleaning.

2. Characteristics

Name	**LumiLys 780**	**MultiLys 780 T_2**	**MultiLys 625 T_2**	**MultiLys 650 T_1**
Size	25 nm 	200 nm 	150 nm 	25 nm 
Shape	Spherical			
Composition	$Cy7@SiO_2$	$Dy_2O_2S@SiO_2$-Cy7	Gd_2O_2S:Eu	$Ru@SiO_2$@DTPA-Gd
Fluorescence color	NIR	NIR	Red	Deep red
Excitation	750 nm	750 nm	340-365 nm	365 / 488 nm
Emission	780 nm	780 nm	625 nm	650 nm
MRI	No	T_2	T_2	T_1
X-Ray	No	Yes	Yes	No
Packaging	3 mg			
Nb labelled cells	$\approx 4.10^6$	$\approx 1.10^6$		
Dilution medium	5% glucose water			

3. How to use cell tracking products

Cell tracking products are supplied as dried powders. Suspension in a mother glucose (5%) solution has to be reconstituted by mixing the dried powder and the medium furnished (glucose 5%), and then sonicated 15 minutes in an ultrasonic bath. This mother suspension is thus at a concentration of 1 $mg.mL^{-1}$ and allows to label around 1 M cells.
This mother suspension must be diluted in water, glucose (5%), or culture medium before cell labelling. Usually dilution around 0,1 mg/ml (x10) gives good results (strong labelling without toxicity). However, the endocytosis phenomenon is very dependent from the cell type and this concentration must be optimized by preliminary trials. Avoid use of phosphate buffer saline which causes aggregation effects.

To ensure a good dispersion of the nanoparticles in the suspension, it is strongly recommended to sonicate the vial for 15 minutes prior to any uses.

4. Cell labeling with cell tracking products

4.1. Reconstitution of the suspension

Cell tracking products are supplied as dried powders, the suspension as to be reconstituted as described in the section “3. How to use cell tracking products”.

4.2. General protocol for cell cultures labeling

- Plate the cells into the culture box following the procedure adapted to your cell type.
- Reconstitute the suspension (mother suspension) by mixing the dried powder and the furnished medium as previously described (see “reconstitution of the suspension”).
- Dilute the mother suspension in sterile culture medium to obtain desired concentration (diluted suspension) (see “How much cell tracking product to use”).

- Remove culture medium from the cells and replace by the same volume of the diluted cell tracking suspension.
- Incubate for 8 to 24 hours depending on the cell line.
- Remove culture medium containing cell tracking suspension and wash 3 times with fresh sterile Phosphate Buffer Saline solution or culture medium.
- Analyze labelling using fluorescence or confocal microscope with appropriate filters (see section "characteristics").
- For *in vivo* injection of the labelled cells, apply your usual procedure.

4.3. In vivo observations

Follow the imaging protocol as recommended by the manufacturer of your imaging system.

For fluorescence imaging, select appropriate excitation and emission filters (see "2. Characteristics").

For MRI, choose appropriate sequence. MultiLys 780 T_2 and MultiLys 625 T_2 products produce good contrast in T_2 and T2* imaging. MultiLys 650 T_1 product gives good contrast in T_1 imaging.

Images acquisition over an extended time period after injection is recommended.

Post-mortem fluorescence imaging can also be done on cytological sampling. *In vivo* tumor targeting products resist to common fixative aldehyde solutions. LumiLys and MultiLys products are not sensible (or very weakly) to photo-bleaching.

4.4. How much cell tracking product to use?

The amount of cell tracking product needed in order to obtain a good cell labeling varies according to the cell type. Prior to any cell labeling experimentation, it is strongly recommended to define adapted cell tracking product amount. It can be realized by using the previous protocol (see "general protocol for cell culture labeling") and varying the concentration of the diluted suspension. Optimized concentration is obtained when a good labeling is observed, with the minimum cellular death.

Usually, 0.1 to 1 ng/cell yields sufficient labeling for studies using fluorescence microscopy. However, usually, MRI and X-ray tomography need higher concentration (around 3 ng/cell). Take care that at very high concentrations, cell tracking products should induce some cytotoxic effects.
Usually, dilution of cell tracking mother solution with culture medium in ratio 1/10 to 1/50 gives good labelling without toxic effect.

If you want track cell types which are poorly sensitive to the endocytosis phenomenon, we may supply LumiLys or MultiLys product conjugated with a transfer protein: please contact us.

Please contact us for any additional advice:
contact@chromalys.fr

5. Additional data

You may consult additional data about LumiLys and MultiLys product by uploading technical sheet for each product: click on the link below.

LumiLys 780
MultiLys 780 T_2
MultiLys 625 T_2
MultiLys 650 T_1