Lysolys Series

LysoLys 525, LysoLys 650

1. Product description

1.1 Introduction

LysoLys probes are silica-based fluorescent nanoparticles designed to visualize and track acidic organelles in living cells, and more particularly lysosomes. They penetrate inside the cells by endocytosis and effectively mark lysosomes at low concentrations. LysoLys are no or weakly cytotoxic, no alkalinizing, allowing studies over several days on living cells. LysoLys probes resist to aldehydic fixatives as well to photo-bleaching. Indeed, encapsulated within the mineral silica shell, used chromophores have outstanding resistance to metabolic and photo-chemical degradation. Two LysoLys products, which can be imaged at two different wavelengths, are available: **LysoLys 525 and LysoLys 650.**

1.2 Product format and storage

LysoLys products are supplied as a dried powder. Mother suspension (1 mg/mL) has to be reconstituted by mixing the dried powder and the medium furnished (water-glucose 5%), and then sonicated in an ultrasonic bath[1].

Dried nanoparticles can be preserved indefinitely at 4°C in absence of light. Nanoparticles in suspension should be used within 7 days and strongly vortexed or sonicated just before use to ensure a good dispersion.

LysoLys products contain no preservatives. Avoid any microbial contamination during use.

1.3 Quality control

LysoLys 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.

2. Characteristics

Name	**LysoLys 525**	**LysoLys 650**
Sub-cellular location	Lysosomes	
Size	25 nm	
Shape	Spherical	
Composition	FITC@SiO2	Ru@SiO2
Emission colour	Green	Red
Excitation	495 nm	365 / 488 nm
Emission	525 nm	650 nm
Molar exctinction coefficient ε	ε = 80 000 $cm^{-1}.M^{-1}$	ε = 13 400 $cm^{-1}.M^{-1}$
Quantum yield	Φ = 0,9	Φ = 0,055
Brightness	72 000	737[2]
Containing	3 mg	
Nb labelled cells	6.10^7 to 6.10^8	
Dilution medium	Glucose 5%, culture medium	

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

[2] Don't be afraid by the weak calculated brilliance for LysoLys 650 dye. The strong stock shift between excitation and emission allow to incorporate a huge amount of Ru complex within the silica matrix without seeing any quenching effect. That means, one LysoLys 650 particle are, as bright, as hundreds of single molecule. Practically, you will have no difficulty to detect this product.

3. How to use LysoLys products

LysoLys products are supplied as a dried powder. Mother suspension in glucose (5%) solution has to be reconstituted by mixing the dried powder and the medium furnished (glucose 5%), and then sonicated for 15 minutes in an ultrasonic bath. As-prepared mother suspension is thus at a concentration of 1 $mg.mL^{-1}$ and allows the labelling of 6.10^7 to 6.10^8 cells.
This mother suspension must be diluted in 5% glucose-water, or culture medium before cell labelling. Dilution around 0.05 mg/ml (x20) or less 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.
Strong lysosome labelling is not always needed. If you want to avoid particle agglomeration inside lysosomes (and at the cell membrane surface) you may dilute your mother solution until 0,005mg/ml (or even less) for a weak but more homogeneous labelling.

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 use.

4. Cell labeling with LysoLys

4.1. Reconstitution of the suspension

LysoLys products are supplied as dried powders, the suspension as to be reconstituted as described in the section "3. How to use LysoLys products".

4.2. General protocol for the cell cultures labeling

- Plate the cells into the culture box following the procedure adapted to you 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 LysoLys product to use").
- Remove culture medium from the cells and replace by the same volume of the diluted LysoLys suspension.
- Incubate for 8 to 24 hours depending on the cell line.
- Remove culture medium containing LysoLys and wash 3 times with fresh sterile Phosphate Buffer Saline solution (or your culture medium).
- Observe the cells using fluorescence or confocal microscope with appropriate filters.

4.3. How much LysoLys product to use?

The amount of LysoLys needed in order to obtain a good lysosomal labeling varies according to the cell type and your need. Prior to any lysosomal labeling experimentation, it is strongly recommended to define adapted LysoLys 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, without cellular death and unwanted LysoLys agglomeration.

Usually, 10 to 100 pg of LysoLys are needed to label 1 cell. Thus concentrations of the diluted suspension in the range of 1 to 50 µg/ml are sufficient.
Note that at high concentrations (over 1 ng/cell) LysoLys should induce some toxic effect.

Please contact us for any additional advice:
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5. References

LysoLys 650 has been designed following a fruitful collaboration with M.J. Menu teams:
Cousinié S.,Mauline L.,Gressier M.,Kandibanda S.R., Datas L., Reber C., Menu M.J., Bulk or surface grafted silylated Ru(ii) complexes on silica as luminescent nanomaterials. (2012) *New Journal of Chemistry*, vol. 36 (n° 6). pp. 1355-1367