ExoSparkler Exosome Protein Labeling Kit-Green
ExoSparkler Exosome Protein Labeling Kit-Red
ExoSparkler Exosome Protein Labeling Kit-Deep Red

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/EX04, EX05, EX06.pdf

General Information

Recent findings suggest that exosomes, a form of extracellular vesicle (EV), contribute to malignant transformation and the metastasis of cancer. Consequently, intercellular communication via exosomes is attracting considerable interest in the scientific community. To shed light on such communication, labeling techniques based on fluorescent dyes have been used. Dojindo's ExoSparkler Protein Dyes are the optimized fluorescent dyes for labeling of exosomal protein. Moreover, three different color options of Dojindo's dyes (Green, Red, and Deep Red) enable application to experiments using multiple labels, such as investigation of exosome localization in intracellular organelles.

Kit Contents

	Labeling Dyes	Filtration Tube
EX04 ExoSparkler Exosome Protein Labeling Kit-Green	Protein Dye-Green x 1	x 5
EX05 ExoSparkler Exosome Protein Labeling Kit-Red	Protein Dye-Red x 1	x 5
EX06 ExoSparkler Exosome Protein Labeling Kit-Deep Red	Protein Dye-Deep Red x 1	x 5

Storage Condition

Store in a cool (0–5°C), dark, and dry place.

Required Equipment and Materials

- Dimethyl sulfoxide (DMSO)
- PBS
- Micropipette
- Microtube
- Centrifuge

Preparation of solutions

Preparation of Protein Dye stock solution

Add 10 µl of DMSO to a tube of Protein Dye-Green, -Red, or -Deep Red and dissolve using a vortex mixer.

Note: Protein Dye-Green is difficult to see because it is present in a small amount and is a colorless foam. Please prepare the Protein Dye-Green stock solution carefully by vortexing with DMSO as described in the protocol.

Note: Store the Protein Dye stock solution at −20°C.

Note: To avoid the effect of repeated freeze/thaw cycles, it is recommended that Protein Dye stock solution be aliquoted in small volumes (e.g., 2 µl each) and then stored frozen.

General Protocol

Preparation of cells

1. Seed the cells on a dish for assaying. Culture the cells at 37°C overnight in a 5% CO_2 incubator.

Labeling of exosome

Table 1. Recommended amount of exosome

Protein amount	1 – 10 µg
Particle count	10 – 100 x 10^8

Note: The above mentioned conditions have been optimized for exosome purification by ultracentrifugation. In case any other exosome purification method is used, the amount of exosome should be further optimized.

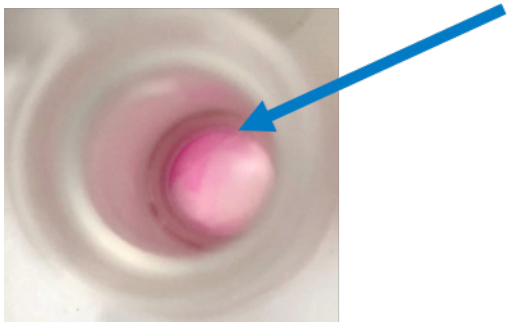
Note: Exosome prepared by polymer-based precipitation cannot be applied with these kits.

1. Resuspend exosome in 100 µl of PBS (recommended amounts are described in Table 1).
2. Add 2 µl of Protein Dye stock solution to the exosome solution and mix using a vortex mixer.
3. Incubate at 37°C for 30 min.
4. Transfer the labeled exosome solution to a filtration tube.
5. Centrifuge at 3000 × g for 5 min at room temperature.
6. Add 100 µl of PBS to the filtration tube.
7. Centrifuge at 3000 × g for 5 min at room temperature.
8. Repeat steps 6 and 7.
9. Centrifuge at 3000 × g for 5 min at room temperature.

Note: Perform an additional 5 min of centrifugation if solution remains on the membrane.

10. Add 50 µl of PBS to the filtration tube and carefully pipette about 10 times to recover the labeled exosome. Transfer the solution to a new microtube.

Note: Do not touch the dyes on the membrane to avoid contamination of excess dyes.



The dyes remain on the membrane
⇒ Carefully pipette to collect the labeled exosomes without touching excess dyes

Addition of labeled exosomes to the cells

11. Remove the culture medium and wash the cells with PBS once.
12. Add culture medium with serum.
13. Add 1–50 µl of labeled exosome in PBS and incubate at 37°C for 1–24 h in a 5% CO_2 incubator.
14. Remove the supernatant and wash the cells with PBS twice.
15. Add culture medium with serum and observe the sample under a fluorescence microscope.

Examples of Use

The effects of exosome amounts and incubation times on the fluorescent signal in HeLa cells.

1. HeLa cells were seeded (1.25 × 10^4 cells/well) on a µ-slide eight-well plate (ibidi) and cultured at 37°C overnight in a 5% CO_2 incubator.
2. The supernatant was removed and the cells were washed once with PBS.
3. A total of 200 µl of DMEM containing 10% FBS was added to each well.
4. The exosome (5 or 10 µg of protein, 50 or 100 × 10^8 particle count) purified by ultracentrifugation (from culture medium of HEK293 adapted to suspension mode) was labeled using ExoSparkler Exosome Protein Labeling Kits, and was added to each well.
   *The protein and particle concentration were quantified by BSA method and nanoparticle tracking analysis, respectively.
5. The cells were incubated at 37°C for 2 or 4 h in a 5% CO_2 incubator.
6. The supernatant was removed and the cells were washed twice with PBS.
7. A total of 200 µl of DMEM containing 10% FBS was added to each well and the cells were observed using a fluorescence microscope.

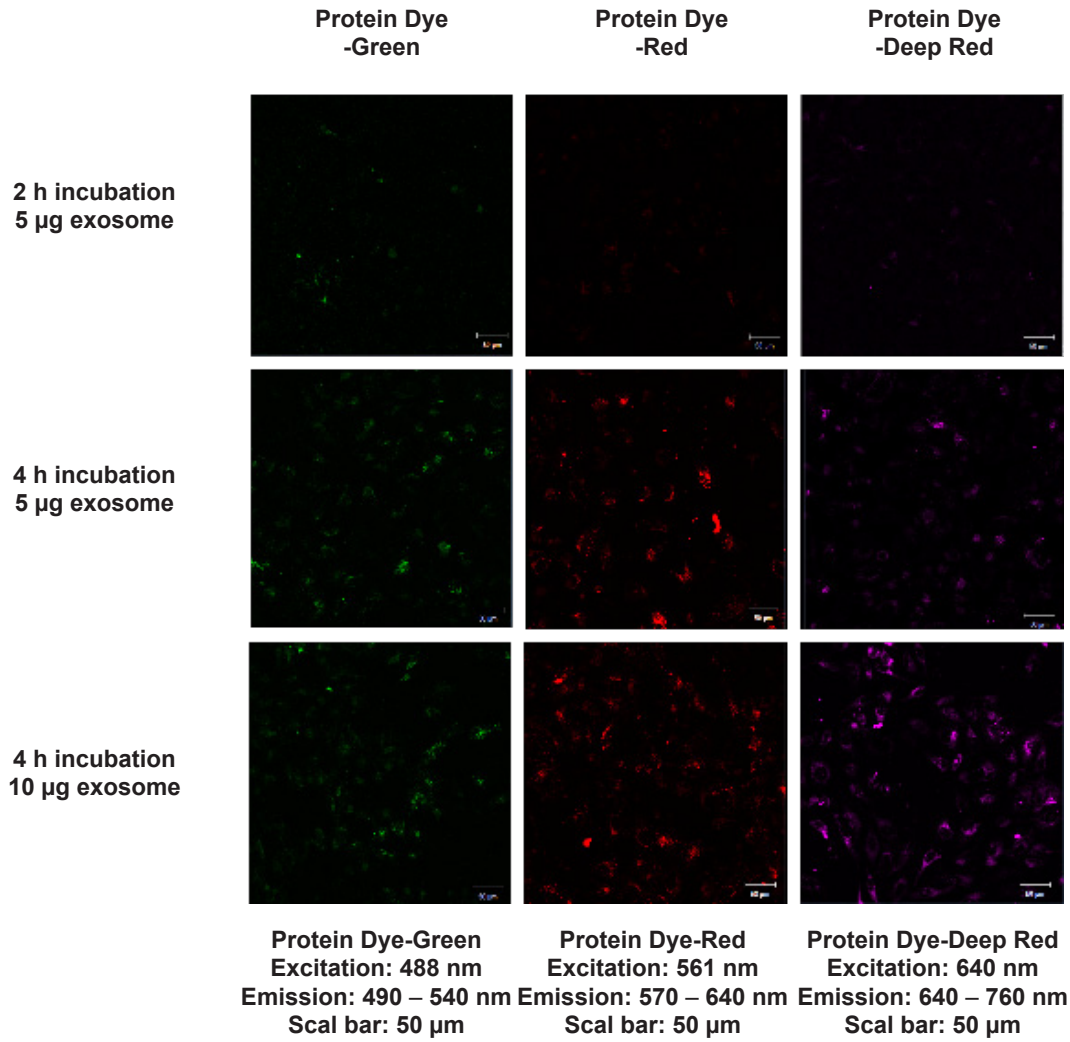


Figure 1. Increment of fluorescent signals depending on exosome amounts and incubation times observed in HeLa cells. These data were obtained by the kind support of Dr. Kazunari Akiyoshi at Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University.

If you need more information, please contact Dojindo technical service.

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