Protocol for DiI-Ac-LDL Uptake

Reagents Included in DiI-Ac-LDL Kit:

		Volume	Storage Temperature
1.	Extracellular Matrix Attachment Solution	2 x 1.5 ml	4ºC
2.	1,1'-dioctadecyl-3,3,3',3'-tetramethyl-indocarbocyanine perchlorate Low Density Lipoprotein (DiI-Ac-LDL), 200µg/ml	120 µl	4ºC
3.	Wash Buffer	20 ml	4ºC
4.	Mounting Solution	600 µl	4ºC

Reagent Required but Not Included in The Kit:

	Endothelial Cell Growth Medium	500 ml	4ºC

Procedure:

Steps 1 to 7 and step 9 should be performed in a sterile Biological Safety Cabinet.
Steps 8 to 18 should be performed in low light to minimize the amount of light exposure.

1. Coat the Lab-Tek chamber slide with Extracellular Matrix Attachment Solution. Add 100 µl per well for 8-well chamber slide or 150 µl per well for 4-well chamber slide.
2. Be sure to keep the Extracellular Matrix Attachment Solution evenly over the entire well surface.
3. Keep the coated chamber slide at 37 ºC for 30 min or at room temperature for 2 hours (overnight is OK).
4. Remove the Extracellular Matrix Attachment Solution by aspiration.
5. Plate endothelial cells on the coated chamber slides at the seeding density of 30,000 cells per well for the 8-well chamber slide, or 60,000 cells per well for the 4-well chamber slide.
6. Grow the cells in Endothelial Cell Growth Medium to 95% confluent.
7. Remove the medium gently. Leave 100 µl of medium in each well of the 8-well chamber slide or 200 µl of medium in each well of the 4-well chamber slide.
8. Spin the DiI-Ac-LDL tube in a microfuge to collect all the reagent droplets that stick to the side of the tube.
9. Add DiI-Ac-LDL directly to the culture medium in each well: Add 5 µl to each well of 8-well chamber slide or 10 µl per well of 4-well chamber slide. This would make the final concentration of DiI-Ac-LDL at 10 µg/ml. Be sure to work quickly in low light to avoid exposing DiI-Ac-LDL to the light.
10. Place the slide in a 37 ºC, 5% CO_2 incubator for 4 hours.
11. Remove the medium by turning the chamber slide upside down onto an absorbent towel in low light to avoid exposing to the light.
12. Wash the cells three times by filling each well with Wash Buffer, then carefully remove the Wash Buffer by turning the chamber slide upside down onto an absorbent towel.
13. Remove the chamber and gasket from the chamber slide with a forceps. Be sure to remove all the gasket cement.
14. Add one drop of Mounting Solution to each chamber area with a dropper.
15. Mount the slide with a cover slip (22 x 50 mm) using a forceps. Slowly and carefully lay down the cover slip without trapping any air bubbles. Work in low light.
16. Hold the side of the slide against an absorbent towel to remove any excess Mounting Solution.
17. Seal the edge of the cover slip with sealant or nail polish.
18. Examine with an epifluorescence microscope using a standard rhodamine excitation/emission filter (red fluorochromic color with 552 nm excitation and 570 nm emission).