DIVERSA

Edificio Emprendia, Campus Sur
15782 Santiago de Compostela
(A Coruña)

www.diversatechnologies.com
info@diversatechnologies.com

DIVERSA FLUOGREEN NANOPARTICLES

Tracking fluorescent DIVERSA for an effective cell internalization

USER PROTOCOL – **#DIV000F1**

Edificio Emprendia, Campus Sur
15782 Santiago de Compostela
(A Coruña)

www.diversatechnologies.com
info@diversatechnologies.com

ABOUT THE NANOPARTICLES

OVERVIEW

DIVERSA is a biocompatible, biodegradable, and cell-friendly technology for enhancing intracellular delivery of small molecules and biomolecules, paving the way towards clinical translation.

DIVERSA FLUOGREEN NANOPARTICLES uses strongly labelled fluorescent nanometric emulsions that are easily internalized by live cells. They can be visualized by a wide variety of platforms (flow cytometry, microplate assays, fluorescence, and confocal microscopy) in less than two hours at Ex/Em = 495/503 nm,

DIVERSA FLUOGREEN NANOPARTICLES can be used as a positive control for cell internalization before testing the efficiency of associated molecules in specific cell lines of interest.

DIVERSA FLUOGREEN NANOPARTICLES can also be used as a positive control to normalize the values obtained with other types of delivery systems, as well as with exosomes.

COMPONENTS

- 1x DIV000F1 vial for reconstitution.
- 1x DIVTECH vial for preparation of each DIVERSA FLUOGREEN NANOPARTICLES.
- 2x Tips for 1 mL micropipette.

STORAGE

Before formulating, store the vials at -20 ºC. Once formulated, storage is recommended at 4 ºC.

FOR RESEARCH USE ONLY. Not for use in humans. Purchase of this product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

- 1 mL micropipette.
- Sterile standard microtubes.
- MilliQ water or any other recommended buffer.
- Ethanol (EtOH) 96%.

CONSIDERATIONS BEFORE STARTING

- The following protocol is optimized for the preparation of 1 mL of DIVERSA FLUOGREEN NANOPARTICLES (starting from one DIV000F1 vial for reconstitution).
- DIVERSA cannot guarantee the optimal characteristics of the final formulation if modifications in the protocol are introduced.
- It is recommended to use DIVERSA FLUOGREEN NANOPARTICLES within the following 60 days.
- DIVERSA FLUOGREEN NANOPARTICLES is stable for 24 h in cell culture media at 37 ºC: DMEM and RPMI (with/without FBS).
- Do NOT use any buffer solution containing Triton-X, SDS or Tween-20 for preparation or manipulation of DIVERSA FLUOGREEN NANOPARTICLES.
- Do NOT freeze DIVERSA FLUOGREEN NANOPARTICLES.
- Do NOT heat up DIVERSA FLUOGREEN NANOPARTICLES.
- Do NOT centrifuge or vortex DIVERSA FLUOGREEN NANOPARTICLES.

FOR RESEARCH USE ONLY. Not for use in humans. Purchase of this product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited.

Edificio Emprendia, Campus Sur
15782 Santiago de Compostela
(A Coruña)

www.diversatechnologies.com
info@diversatechnologies.com

DIVERSA FLUOGREEN NANOPARTICLES
PROTOCOL

1. Reconstitute the DIV000F1 vial with 100 µL of EtOH. Pipette up and down gently for mixing the lipids trying to recover all of them from the vial wall and keep the suspension in the vial.
2. Add 900 µL of ultrapure water into the DIVTECH vial or, alternatively, a buffer solution suggested in Table 1 (Recommendations of Use and Technical Notes).
3. Transfer the whole volume from the DIV000F1 vial to the DIVTECH vial using a micropipette and the 1 mL micropipette tip provided.

IMPORTANT: Before adding the lipids from DIV000F1 vial to the DIVTECH vial, set the micropipette at the maximum volume to have dead air volume in the tip for mixing in a faster and vigorous way. Then, place the 1 mL micropipette tip into the buffer solution of DIVTECH vial, and pipette up and down for 30 seconds, avoiding any spillage.

The DIVERSA FLUOGREEN NANOPARTICLES now ready-to-use for *in vitro* and *in vivo* experiments. Alternatively, keep it at 4 ºC and use it in the following 60 days.

Note: for *in vitro* experiments, the adherent cells must be between 70-80% of confluency on the day of the experiment. However, optimizations should be required depending on the cell type.

FOR RESEARCH USE ONLY. Not for use in humans. Purchase of this product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited.

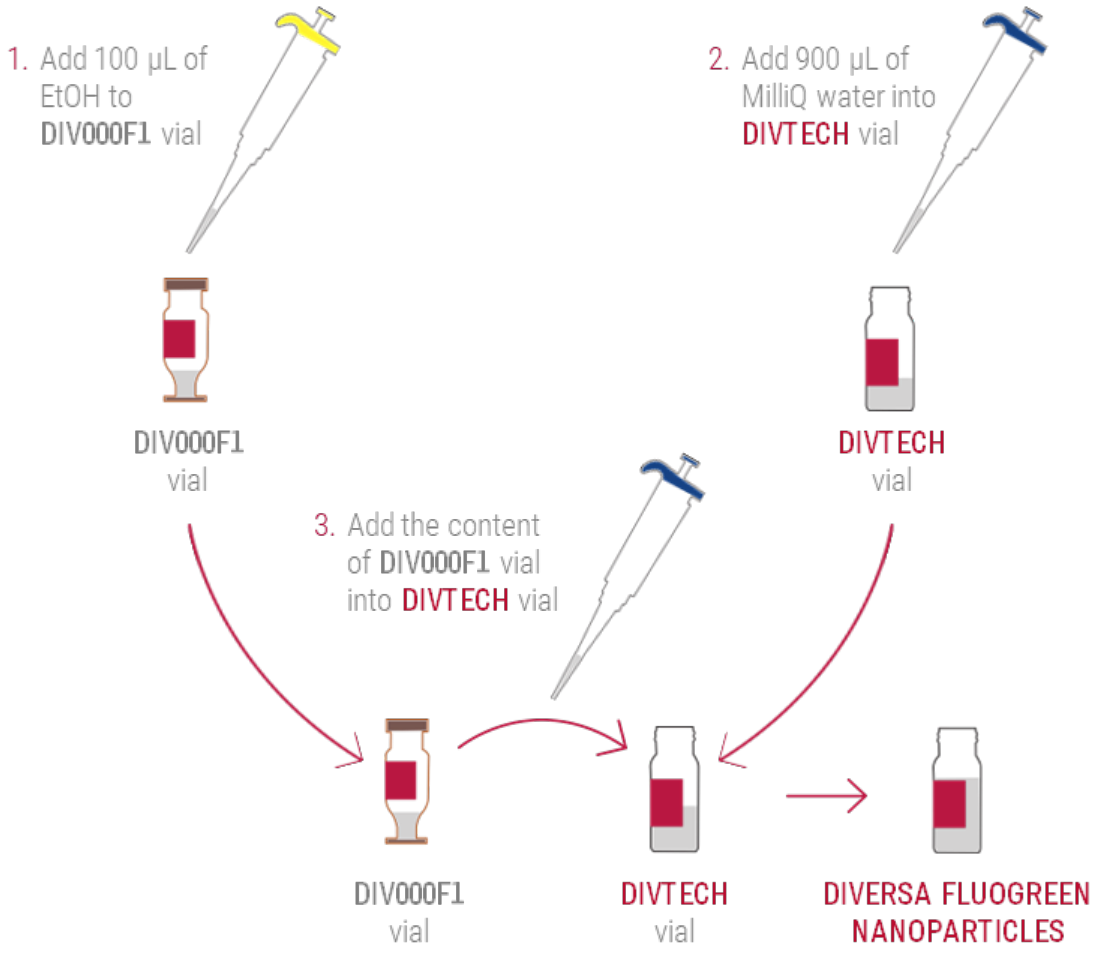

Figure 1. DIVERSA FLUOGREEN NANOPARTICLES protocol.

FOR RESEARCH USE ONLY. Not for use in humans. Purchase of this product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited.

EXAMPLE OF UPTAKE ASSAY PROTOCOL

1. Seed the recommended number of the cells in a 6-well plate the day before of the uptake experiment for FACS analysis.

   Note: the cell density should be determined for each cell type and well plate.

2. Reconstitute the DIV000F1 vial with 100 µL of EtOH. Pipette up and down gently for mixing the lipids trying to recover all of them from the vial wall and keep the suspension in the vial.
3. Add 900 µL of ultrapure water into the DIVTECH vial or, alternatively, a buffer solution suggested in Table 1 (Recommendations of Use and Technical Notes).
4. Transfer the whole volume from the DIV000F1 vial to the DIVTECH vial using a micropipette and the 1 mL micropipette tip provided.

   IMPORTANT: Before adding the lipids from DIV000F1 vial to the DIVTECH vial, set the micropipette at the maximum volume to have dead air volume in the tip for mixing in a faster and vigorous way. Then, place the 1 mL micropipette tip into the buffer solution of DIVTECH vial, and pipette up and down for 30 seconds, avoiding any spillage.

5. Add 960 µL of fresh cell cultured medium supplemented with 10% (v/v) of FBS and, if necessary, 1 % (v/v) of antibiotics.
6. Add the DIVERSA FLUOGREEN NANOPARTICLES to a final volume of 1 mL.

   Note: The final concentration of the DIVERSA FLUOGREEN NANOPARTICLES is calculated considering the final volume of 1 mL.

7. Incubate the cells for 2-4 hours at 37 ºC.

   Note: Depending on the type of readout assay performed, incubation times may influence delivery efficiency.

7. After incubation time: remove the cell culture medium with the DIVERSA FLUOGREEN NANOPARTICLES and, carefully, wash the cells twice with PBS 1X buffer and remove it.

   The DIVERSA FLUOGREEN NANOPARTICLES is efficiently internalized inside the cells.

   Note: we recommend wash the cells with PBS 1X buffer containing calcium and magnesium ions to avoid maximum detachment of living cells.

8. Adjust the concentration for the analysis using the flow cytometer and the following parameters: wavelength of excitation at 595 nm, wavelength of emission at 503 nm.

   Note: we recommend analysis on the same day of the experiment. However, for extended storage (> 16 h), we recommend resuspend the cells in 4% (v/v) of paraformaldehyde to prevent cell deterioration.

FOR RESEARCH USE ONLY. Not for use in humans. Purchase of this product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited.

Edificio Emprendia, Campus Sur
15782 Santiago de Compostela
(A Coruña)
www.diversatechnologies.com
info@diversatechnologies.com

RECOMMENDATIONS OF USE & TECHNICAL NOTES

Table 1. Suggested buffer for DIVTECH vial.

BUFFER SOLUTION	CONCENTRATION
Ultrapure water	N/A
PBS	2-50 mM
NaCl	150 mM
HEPES	10-25 mM
DPBS	1X

Table 2. Recommended volumes for cell culture.

Cell culture vessel	Volume of **DIVTECH**	Volume of medium	Final volume/well
100 cm	200 µL	4,8 mL	5 mL
6-well	40 µL	960 mL	1 mL
12-well	20 µL	996 µL	500 µL
24-well	10 µL	240 µL	250 µL
96-well	4 µL	96 µL	100 µL

FOR RESEARCH USE ONLY. Not for use in humans. Purchase of this product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited.

FREQUENTLY ASKED QUESTIONS

QUESTION	ANSWER
What is the concentration of the fluorophore in DIV000F1?	The concentration of the fluorophore is 4 µg/mL in the final DIVERSA FLUOGREEN NANOPARTICLES.
How do I concentrate the formulation?	If necessary, the 1 mL of DIVERSA FLUOGREEN NANOPARTICLES can be concentrated by using a SpeedVac or Rotavap in mild conditions (avoid surpassing 35 °C or drying out the samples). Samples can be concentrated up to 4-fold its original volume (i.e., to a final volume 250 µL).
Can I filter the formulation?	Yes, if necessary, DIVERSA FLUOGREEN NANOPARTICLES can be filtered using 0.22 µm filters of PES membrane.
Can I use buffers other than MilliQ water?	Yes, please check Table 1 for other recommended buffers.
How can I measure the size of the final formulation?	Diameter size can be measured by Dynamic Light Scattering (DLS) analysis adding to the cuvette 10 µL of DIVERSA FLUOGREEN NANOPARTICLES with 990 µL of MilliQ water.
Can I use DIVERSA FLUOGREEN NANOPARTICLES for ***in vivo*** studies?	Yes, DIVERSA FLUOGREEN NANOPARTICLES can be used *in vivo*. For specific recommendations and a customized and optimized prototype, contact DIVERSA.

ONLINE RESOURCES

Visit our website www.diversatechnologies.com for further information.

FOR RESEARCH USE ONLY. Not for use in humans. Purchase of this product does not include any right to resell or transfer, either as a stand-alone product or as a component of another product. Any use of this Product for purposes other than research is strictly prohibited.