DIVERSA

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

www.diversatechnologies.com
info@diversatechnologies.com

DIVERSA FLUOGREEN CELL INTERNALIZATION NANOPARTICLES

Tracking fluorescent DIVERSA nanoparticles to ensure efficient cell internalization

USER PROTOCOL – **#DIV000F1**

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ABOUT THE NANOPARTICLES

OVERVIEW

DIVERSA FLUOGREEN NANOPARTICLES are fluorescently labeled and biocompatible nanoparticles that are efficiently internalized by live cells. They can be tracked by a wide variety of platforms (flow cytometry, microplate readers, fluorescence, and confocal microscopy) at Ex/Em = 495/503 nm.

DIVERSA FLUOGREEN NANOPARTICLES can be used as a positive control for cell internalization studies and cell tracking in specific cell lines.

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 DIVERSA FLUOGREEN NANOPARTICLES.
- 2x Tips for 1 mL micropipette.

STORAGE

Before formulating, store the vials at -20 ºC. Once formulated, the preparation should be stored at 2-8 ºC.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

- 1 mL micropipette.
- 1.5 mL sterile microtubes.
- Ultrapure water or any other recommended solution (Table 1).
- Ethanol (EtOH) 96%.

CONSIDERATIONS BEFORE STARTING

- The following protocol is optimized for the preparation of 1 mL of DIVERSA FLUOGREEN NANOPARTICLES.
- DIVERSA cannot guarantee the optimal formulation performance if any modifications are made to the protocol.
- It is recommended to use DIVERSA FLUOGREEN NANOPARTICLES within 60 days of preparation for optimal performance.
- DIVERSA FLUOGREEN NANOPARTICLES are stable in cell culture media under the following tested conditions: for at least 24 h at 37 ºC in DMEM and RPMI, with or without FBS.
- Do NOT use any solution containing Triton-X, SDS or Tween-20 for preparation and manipulation of DIVERSA FLUOGREEN NANOPARTICLES.
- Do NOT freeze DIVERSA FLUOGREEN NANOPARTICLES.
- Do NOT heat over 90 ºC DIVERSA FLUOGREEN NANOPARTICLES.

DIVERSA FLUOGREEN NANOPARTICLES

PROTOCOL

1. Add 100 µL of EtOH in the **DIV000F1** vial. Gently pipette up and down.
2. Add 900 µL of ultrapure water, or a recommended solution (see Table 1), to the **DIVTECH** vial.
3. Transfer the entire volume from the **DIV000F1** vial to the **DIVTECH** vial using a 1 mL micropipette and the provided tip.

   Note: Before adding the volume from the **DIV000F1** vial into the **DIVTECH** vial, set the micropipette at the maximum volume, and add the solution with a sudden, vigorous downward motion. Pipette up and down for 5-10 seconds with confidence.

   The **DIVERSA FLUOGREEN NANOPARTICLES** are now ready to use or can be stored at 2-8 ºC for up to 60 days.

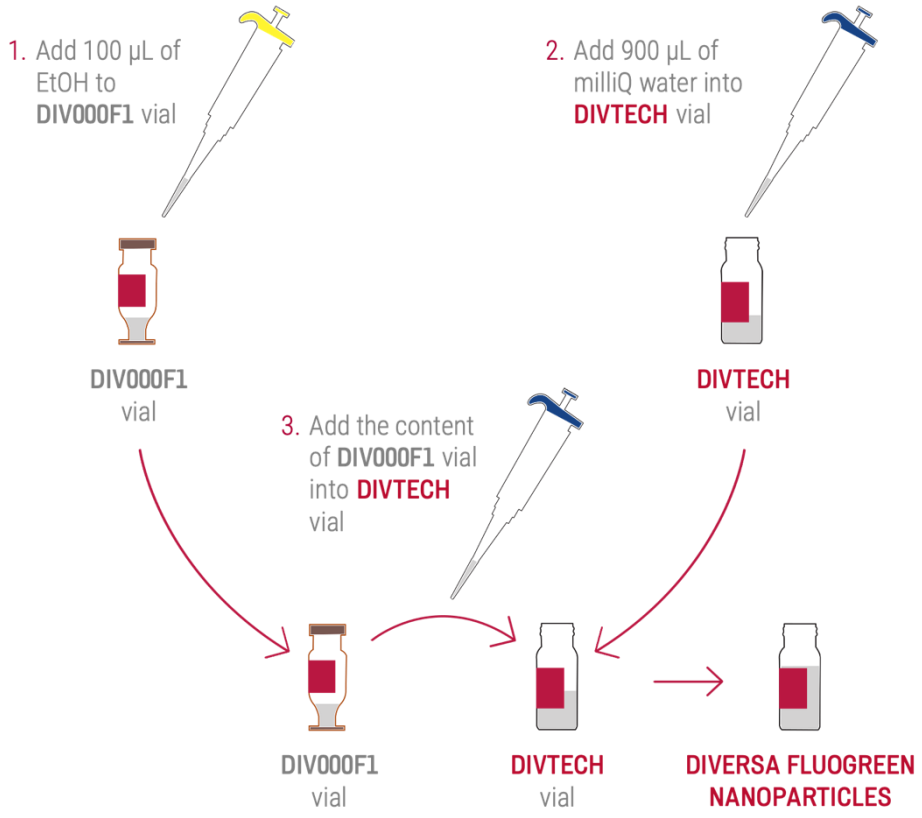

Figure 1. **DIVERSA FLUOGREEN NANOPARTICLES** protocol.

EXAMPLE OF CELL INTERNALIZATION ASSAY PROTOCOL

1. Seed the appropriate number of the cells in a 6-well plate the day before the uptake experiment to analyze by FACS.

   Note: For *in vitro* experiments, adherent cells must be between 70-80% confluency on the day of the experiment. Optimization may be necessary depending on the cell type.

2. Add 960 µL of fresh cell cultured medium and 40 µL of DIVERSA FLUOGREEN NANOPARTICLES.

   $Note_1$: If different well plate formats are used, adjust the volumes accordingly (see suggestions in Table 2).

   $Note_2$: For higher fluorescence intensity, you can increase the volume of DIVERSA FLUOGREEN NANOPARTICLES. We recommend starting with the suggested volume and, if needed, adding more nanoparticles based on your results.

3. Incubate the cells for 4 hours at 37 ºC protected from light.

   Note: Optimization can also be performed on incubation time to maximize cell internalization and improve results.

4. After incubation, remove the cell culture medium containing DIVERSA FLUOGREEN NANOPARTICLES and carefully wash the cells twice with DPBS 1X buffer. Then, remove the buffer.

   Note: Use DPBS 1X buffer containing calcium and magnesium ions to prevent detachment of living cells.

5. Trypsinize the cells to prepare a cell suspension for FACS analysis, or prepare the sample for evaluation by fluorescence or confocal microscopy. Ensure that the cells and preparations are protected from light to maintain the fluorescence of DIVERSA FLUOGREEN NANOPARTICLES.

6. Set the equipment parameters for with an excitation wavelength of 495 nm and an emission wavelength of 503 nm. Alternatively, use the closest available channels if the equipment is pre-configured.

   Note: We recommend performing the analysis on the same day of the experiment. Alternatively, cells can be fixed using paraformaldehyde.

RECOMMENDATIONS OF USE & TECHNICAL NOTES

Table 1. Suggested solutions for DIVTECH vial.

SOLUTION	CONCENTRATION
Ultrapure water (preferred)	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 **DIVERSA NANOPARTICLES**	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

FREQUENTLY ASKED QUESTIONS

QUESTION	ANSWER
What is the concentration of the fluorophore in DIV000F1?	The fluorophore concentration is 4 µg/mL in the final DIVERSA FLUOGREEN NANOPARTICLES.
Is the green fluorochrome the only available option?	Currently, we just offer the green fluorochrome, but DIVERSA can provide custom formulations with alternative dyes such as Cy5, Cy7.5, and others.
Is fluorochrome pH-sensitive?	No, it is stable across a wide pH range.
How stable is the fluorescence signal of DIVERSA FLUOGREEN NANOPARTICLES?	Green fluorescence remains stable for up to 1 year at -20 ºC prior to formulation. After preparation, it is stable for at least 7 days (maximum tested time) when stored at 2-8 ºC, protected from light. In live cells, the signal can be tracked for at least 7 days.
Does the fluorescence affect biological activity?	No, it does not. The fluorescence comes from molecules covalently linked to the lipids and does not interfere with the biological activity of the cells.
How can I concentrate the formulation?	DIVERSA FLUOGREEN NANOPARTICLES can be concentrated using an Amicon Ultra Centrifugal Filter, SpeedVac or Rotavap in mild conditions (avoid surpassing 35 °C or drying out the samples). Samples can be concentrated up to 4-fold (to a final volume of 250 µL).
Can I filter the formulation?	Yes, DIVERSA FLUOGREEN NANOPARTICLES can be filtered using a 0.22 µm PES membrane filter if needed.
Can I use solutions other than MilliQ water?	Yes, refer to Table 1 for other recommended solutions.
How can I measure the size of the final formulation?	Measure particle size using Dynamic Light Scattering (DLS) by 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?	DIVERSA FLUOGREEN NANOPARTICLES can be used for cell or organ analysis by flow cytometry or confocal microscopy, and we offer customized solutions to adjust fluorophore concentration as needed. For whole-body imaging, DIVERSA provide customized reagents labeled with fluorophores such as Cy7.5, tailored to your experimental settings.

ONLINE RESOURCES

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

Click here to watch the video of the FORMULATION STEP.

CHANGELOG

Version	Date	Change Description
1.0	1 MAR 2022	Initial release of the protocol.
2.0	1 OCT 2024	Improved readability and flow of the protocol for ease of use.