

## **DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES**

Tracking intracellular delivery of a broad range of anionic peptides

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### **USER PROTOCOL – #DIV042F1**

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## ABOUT THE NANOPARTICLES

### OVERVIEW

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**DIVERSA ANIONIC PEPTIDE DELIVERY NANOPARTICLES** are biocompatible, biodegradable, and cell-friendly technology designed to enhance the intracellular and extracellular delivery of anionic peptides, paving the way for clinical translation.

**DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES** are ideal for efficiently associating anionic peptides ( $\text{pH} > \text{pI}$ ) primarily due to the electrostatic interactions between the positively charged nanoparticles and the negatively charged peptides. These nanoparticles are easily internalized by cells and can penetrate more complex structures, such as 3D cell cultures and organoids, while supporting extracellular applications. Additionally, they can be adapted to various routes of administration for evaluation in animal models, maximizing targeted biodistribution and enhancing their therapeutic effect.

**DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES** uses strongly labelled fluorescent nanometric emulsions that are easily internalized by live cells that can be visualized by a wide variety of platforms (flow cytometry, microplate assays, fluorescence, and confocal microscopy) in less than 2 hours at  $\text{Ex/Em} = 495/503 \text{ nm}$ .

**DIVERSA FLUOGREEN** formulation can be used as a positive control for cell internalization and for testing the efficiency of associated proteins in specific cell lines of interest.

### COMPONENTS

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- 1x **DIV042F1** vial for reconstitution.
- 1x **DIVTECH** vial for preparation of **DIVERSA FLUOGREEN PEPTIDE DELIVERY NANOPARTICLES**.
- 2x Tips for 1 mL micropipette.

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Shipping temperature may differ from storage temperature. This does not alter the performance of the product.

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## STORAGE

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Before formulating, store the vials at -20 °C. Once formulated, the preparation should be stored at 2-8 °C for up to 60 days without the peptide, or up to 2 days with the associated peptide.

## EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

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- 1 mL micropipette.
- 20-200 µL micropipette.
- 0.6 mL microtubes.
- 1.5 mL microtubes.
- Ultrapure water
- Ethanol (EtOH) 96%
- Anionic peptide/s of interest

## CONSIDERATIONS BEFORE STARTING

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- The following protocol is designed for anionic peptide delivery. The isoelectric point (pI) of the peptide/s must be **lower than the buffer pH** to ensure efficient association with the nanoparticles.
- 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 (prior to association of the peptide) within 60 days of preparation for optimal performance.
- After anionic peptide association, it is recommended to use **DIVERSA FLUOGREEN PEPTIDE** nanoparticles within 48 hours for optimal performance.
- **DIVERSA FLUOGREEN PEPTIDE** nanoparticles are stable in cell culture media under the following tested conditions: at least 24 h at 37 °C in DMEM and RPMI, supplemented with 10% (v/v) of FBS and 1% (v/v) of penicillin/streptomycin.
- Do NOT use any buffer solution containing Triton-X, SDS or Tween-20 for the preparation **DIVERSA FLUOGREEN PEPTIDE** nanoparticles.
- Once formulated, do NOT freeze **DIVERSA FLUOGREEN PEPTIDE** nanoparticles.
- Do NOT heat over 90 °C **DIVERSA FLUOGREEN PEPTIDE** nanoparticles.

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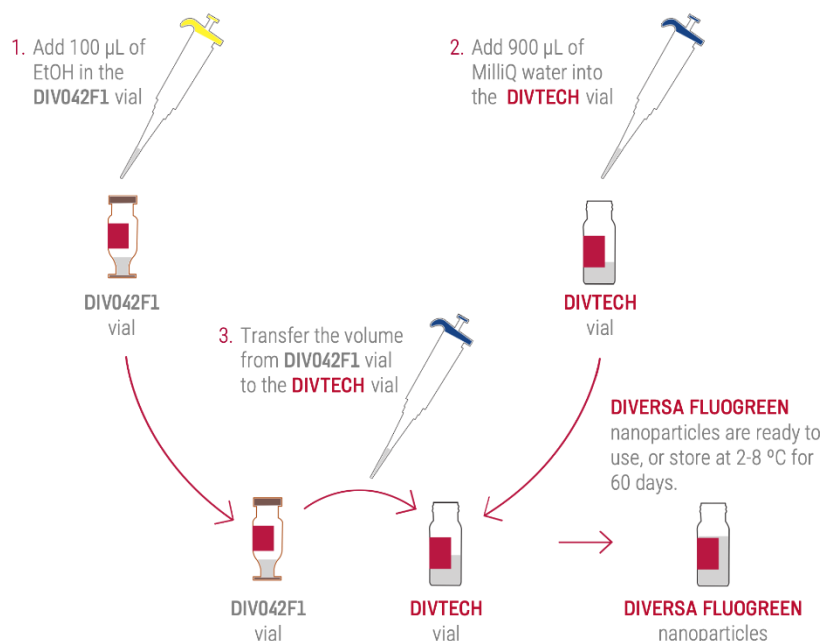
## DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES FORMULATION STEP

Click [here](#) to view the video on the preparation of **DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES**.

1. Add 100  $\mu\text{L}$  of EtOH into the **DIV042F1** vial. Gently pipette up and down.
2. Add 900  $\mu\text{L}$  of ultrapure water into the **DIVTECH** vial.
3. Transfer the entire volume from **DIV042F1** vial to the **DIVTECH** vial using a 1 mL micropipette and the provided tip.

**Note:** Before adding the volume from **DIV042F1** vial to **DIVTECH** vial, set the micropipette at the maximum volume, and add the solution with a sudden, vigorous downward motion. Pipette up and down 5-10 seconds with confidence.

The **DIVERSA FLUOGREEN** nanoparticles are now ready to use or can be stored at 2-8  $^{\circ}\text{C}$  for up to 60 days. They can be used in the [Peptide Association Step](#) either all at once for the formulation of a single peptide or split into fractions for different timepoints or peptides, depending on the experimental needs.



**Figure 1. DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES: FORMULATION STEP.**

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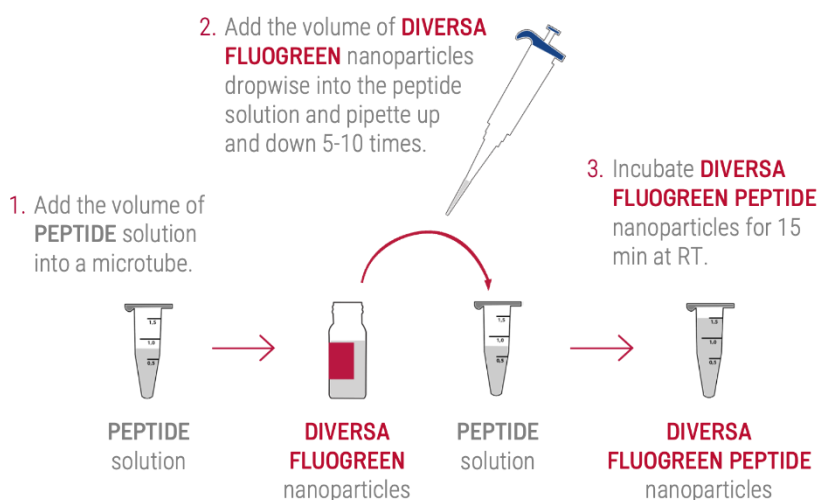
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## PEPTIDE ASSOCIATION STEP

**Note:** Determine first the amount of anionic peptide needed for your experiment. Based on that amount, use **Table 1. of Tables and Technical Notes** to identify a suitable combination of peptide quantity and nanoparticle volume according to the recommended 1.5:1 (v/v) nanoparticles to peptide solution

1. Add the required volume of the **PEPTIDE** solution (refer to **Table 1. of Tables and Technical Notes**) into a microtube.
2. Add the recommended volume of **DIVERSA FLUOGREEN** nanoparticles (refer to **Table 1. of Tables and Technical Notes**) gently and dropwise into the **PEPTIDE** solution. Use a 1.5:1 (v/v) volume ratio of **DIVERSA FLUOGREEN** nanoparticles to the **PEPTIDE** solution. Pipette up and down 5-10 times with confidence.
3. Incubate the **DIVERSA FLUOGREEN PEPTIDE** nanoparticles at room temperature (RT) for 15 min. Agitation is not required.

The **DIVERSA FLUOGREEN PEPTIDE** formulation is now ready to use or can be stored at 2-8 °C for up 2 days.



**Figure 2. DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES: PEPTIDE ASSOCIATION STEP**

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## PEPTIDE DELIVERY

### EXAMPLE PROTOCOL

The following protocol has been validated using L-Glutathione as a peptide model (characteristics: 3 amino acids (L-cysteine, glycine and L-glutamate), pI: 5.93, net charge: highly negative at physiological pH).

1. Seed the recommended number of cells in a 12-well plate with 500  $\mu$ L of complete medium the day before the experiment. Cells should be 70-80% confluent on the day of the experiment.

**Note:** For *in vitro* experiments, the adherent cells must be between 70-80% confluent on the day of the experiment. However, optimizations should be performed depending on the cell type and the length of the experiment.

2. Formulate **DIVERSA FLUOGREEN** nanoparticles following the steps 1-3 of the FORMULATION STEP.
3. Prepare a stock solution of L-Glutathione at a known concentration (e.g., 1 mg/mL). From this solution, take the volume corresponding to 25  $\mu$ g (i.e., 25  $\mu$ L) and dilute it up to 40  $\mu$ L with MilliQ water. Then, add the 60  $\mu$ L of **DIVERSA FLUOGREEN** nanoparticles dropwise onto the **PEPTIDE** solution following the steps 1-3 of the PEPTIDE ASSOCIATION STEP. The **DIVERSA FLUOGREEN L-Glutathione** nanoparticles are ready to use or can be stored at 2-8  $^{\circ}$ C for up to 2 days.
4. Carefully remove the culture medium from each well. Add 900  $\mu$ L of fresh complete medium, then add 100  $\mu$ L of the **DIVERSA L-Glutathione** nanoparticles prepared in Step 3 (containing 25  $\mu$ g of peptide), bringing the final volume to 1 mL per well.
5. Incubate the cells at 37  $^{\circ}$ C in a CO<sub>2</sub> incubator under standard conditions for at least 2-4 hours.

**Note:** Depending on the type of readout assay performed, shorter or longer incubation time may influence delivery efficiency.

6. After incubation, remove the medium, wash the cells twice with DPBS 1X and proceed with the appropriate assay for the desired readout (FACS analysis, fluorescent/confocal microscopy, or a plate reader).

**Note:** We recommend washing the cells with DPBS1X buffer containing calcium and magnesium ions to avoid maximum detachment of living cells.

The **DIVERSA FLUOGREEN PEPTIDE** nanoparticles are efficiently internalized inside the cells. Adjust the equipment settings to visualize **DIVERSA FLUOGREEN PEPTIDE** nanoparticles, setting the excitation wavelength to 495nm and emission wavelength to 503 nm.

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## TABLES AND TECHNICAL NOTES

**Table 1.** Recommended peptide amounts and corresponding volumes of buffer and nanoparticles for the preparation of **DIVERSA FLUOGREEN PEPTIDE** nanoparticles.

Amount of ANIONIC PEPTIDE (µg)	Volume of Peptide solution (µL)	Volume of DIVERSA FLUOGREEN NANOPARTICLES (µL)
0.1-10	4-10	6-15
10-25	10-40	15-60
25-50	40-80	60-120
50-100	80-167	120-250
100-200	167-333	250-500
> 200	333-667	500-1000

**Note:** You may choose any combination within these ranges based on the amount of peptide required for your experiment, maintaining a 1.5:1 (v/v) volume ratio of **DIVERSA FLUOGREEN** nanoparticles to the peptide solution

**Example:** If your experiment requires 40 µg of peptide, you may prepare it in a solution of 60 µL of buffer and add 90 µL of nanoparticles to maintain the 1.5:1 (v/v) ratio.

**Table 2.** Suggested volumes for **DIVERSA FLUOGREEN PEPTIDE** nanoparticles to cell culture medium depending on the cell culture vessel.

Cell culture vessel	Volume of DIVERSA FLUOGREEN PEPTIDE nanoparticles (µL)
96-well	10-25
24-well	25-100
12-well	100-200
6-well	200-400
60 mm	400-800
100 mm	800-1000

**Note:** You may optimize the volume within these ranges depending on your cell type, confluency, and assay sensitivity.

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## FREQUENTLY ASKED QUESTIONS

QUESTION	ANSWER
What is the amount of the fluorophore in DIV042F1?	The fluorophore amount is 4 µg.
Is the fluorochrome pH sensitive?	No, it is stable across a wide pH range.
Does the fluorochrome affect the biological activity	No, it does not. The fluorochrome is covalently linked to the lipids and does not interfere with the biological activity of the cells
Can I filter the formulation?	Yes, <b>DIVERSA FLUOGREEN PEPTIDE</b> nanoparticles can be filtered using 0.22 µm PES membrane filters if needed.
How can I measure the size of the final formulation?	Diameter size can be measured using Dynamic Light Scattering (DS) analysis.
Can I use <b>DIVERSA FLUOGREEN PEPTIDE</b> nanoparticles for <i>in vivo</i> studies?	Yes, <b>DIVERSA FLUOGREEN PEPTIDE</b> nanoparticles can be used <i>in vivo</i> . <b>DIVERSA</b> can provide customized reagents labeled with fluorophores such as Cy5 or Cy7.5, tailored to your experimental settings. For a customized and optimized prototype, contact <b>DIVERSA</b> .
How stable is the signal from the <b>DIVERSA FLUOGREEN PEPTIDE</b> nanoparticles?	Upon formulation, you can use it for up to 7 days if it is stored at 4 °C and protected from light. Regarding stability upon addition to cell cultures, we have tracked it in live cells for up to 8 days.
How do I concentrate on the formulation?	<b>DIVERSA FLUOGREEN PEPTIDE</b> nanoparticles can be concentrated using an Amicon Ultra Centrifugal Filter, SpeedVac or Rotavap in mild conditions (avoid overpassing 35 °C or drying out the samples). Samples can be concentrated down to a quarter of their original volume (e.g., to a final volume of 250 µL).

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## ONLINE RESOURCES

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Visit our website [www.diversatechnologies.com](http://www.diversatechnologies.com) for further information. Click [here](#) to watch the video of the protocol guide.

## CHANGELOG

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Version	Date	Change Description
1.0	1 MAR 2022	Initial release of the protocol.
2.0	1 AUG 2025	Added volume ratio guidance and revised instructions to minimize user-side optimization. Updated example protocol accordingly.

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