

# DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES

Tracking intracellular delivery of anionic peptides

---

## USER PROTOCOL – #DIV042F1

|  |          |
|--|----------|
| <b>ABOUT THE NANOPARTICLES .....</b>   | <b>1</b> |
| OVERVIEW .....   | 1        |
| COMPONENTS .....   | 1        |
| STORAGE .....  | 1        |
| EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED .....                          | 1        |
| CONSIDERATIONS BEFORE STARTING .....   | 2        |
| <b>DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES ..</b>               | <b>2</b> |
| PROTOCOL.....  | 3        |
| EXAMPLE OF TITYUS STIGMURUS ANIONIC PEPTIDE (TanP)<br>ASSOCIATION PROTOCOL ..... | 5        |
| EXAMPLE OF UPTAKE ASSAY PROTOCOL .....   | 6        |
| <b>OPTIMIZATION GUIDELINES.....</b>  | <b>7</b> |
| <b>RECOMMENDATIONS OF USE AND TECHNICAL NOTES .....</b>                          | <b>8</b> |
| <b>FREQUENTLY ASKED QUESTIONS .....</b>  | <b>9</b> |
| <b>ONLINE RESOURCES .....</b>  | <b>9</b> |

## ABOUT THE NANOPARTICLES

### OVERVIEW

---

**DIVERSA** is a biocompatible, biodegradable, and cell-friendly technology for enhancing intracellular delivery of anionic peptides, paving the way towards clinical translation.

**DIVERSA FUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES**, based on cationic lipids, is suitable for an efficient association of your anionic peptides (pH > pI) mainly due to electrostatic interactions.

### COMPONENTS

---

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

### STORAGE

---

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

---

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

**DIVERSA TECHNOLOGIES S.L.** | Edificio Emprendia, Campus Sur, 15782, Santiago de Compostela, Spain.

Technical support: email: [info@diversatechnologies.com](mailto:info@diversatechnologies.com) | [www.diversatechnologies.com](http://www.diversatechnologies.com)

**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%.
- Anionic peptide of interest.

## CONSIDERATIONS BEFORE STARTING

---

- The following protocol is directed for anionic peptides where the isoelectric point (pI) must be higher than the pH of the buffer.
- The following protocol is optimized for the preparation of 1 mL of **DIVERSA FLUOGREEN NANOPARTICLES** (starting from one DIV042F1 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 60 days.
- **DIVERSA FLUOGREEN NANOPARTICLES** is compatible with supplemented cell culture media at 37 °C: DMEM, RPMI.
- Do NOT use any buffer solution containing Triton-X, SDS or Tween-20 for the preparation or manipulation of **DIVERSA FLUOGREEN/DIVERSA FLUOGREEN-PEPTIDE NANOPARTICLES**.
- Do NOT freeze **DIVERSA FLUOGREEN/DIVERSA FLUOGREEN-PEPTIDE NANOPARTICLES**.
- Do NOT heat up **DIVERSA FLUOGREEN/DIVERSA FLUOGREEN-PEPTIDE NANOPARTICLES**.
- Do NOT centrifuge or vortex **DIVERSA FLUOGREEN/DIVERSA FLUOGREEN-PEPTIDE NANOPARTICLES**.

---

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

**DIVERSA TECHNOLOGIES S.L.** | Edificio Emprendia, Campus Sur, 15782, Santiago de Compostela, Spain.

Technical support: email: [info@diversatechnologies.com](mailto:info@diversatechnologies.com) | [www.diversatechnologies.com](http://www.diversatechnologies.com)

**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.

## DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES PROTOCOL

---

1. Reconstitute the **DIV042F1** vial with 100  $\mu$ L of EtOH. Pipette up and down gently for mixing the lipids trying to recover all of them from the wall of the vial and keep the suspension in the vial.
2. Add 900  $\mu$ L 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 **DIV042F1** vial to the **DIVTECH** vial using a micropipette and the 1 mL micropipette tip provided.

**IMPORTANT:** Before adding the lipids from **DIV042F1** vial to **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** is now ready for the association of the anionic peptide/s. Alternatively, keep it at 4 °C and use it in the following 60 days.

4. Add the **DIVERSA FLUOGREEN NANOPARTICLES** gently and dropwise into the anionic peptide solution and pipette up and down gently. Recommended volumes are provided in [Table 2](#).

**Note:** we recommend using peptide stock concentration at 4 mg/mL to increase the reproducibility.

5. Incubate the mixture for 15 min at room temperature (RT). Agitation is not required.

The **DIVERSA FLUOGREEN-PEPTIDE NANOPARTICLES** is now ready-to-use. Alternatively, keep it at 4 °C and use it in the following 2 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 (see [Table 3](#) in Recommendations of Use and Technical Notes).

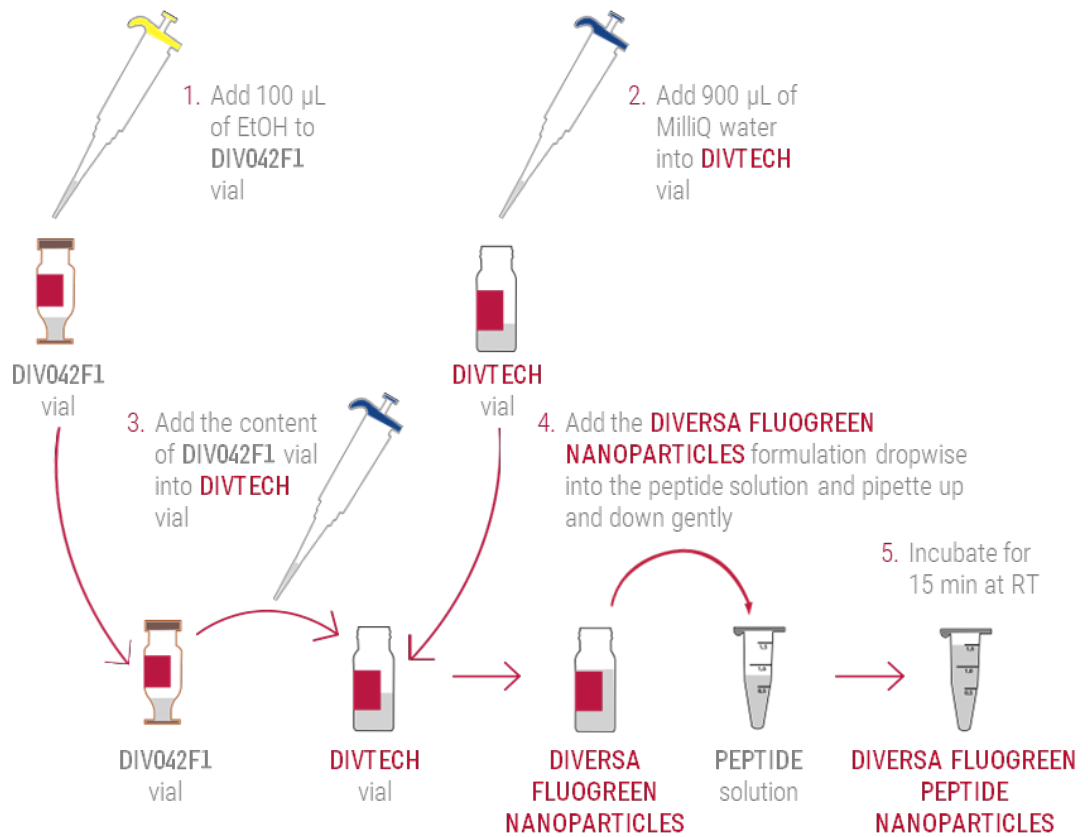
---

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

**DIVERSA TECHNOLOGIES S.L.** | Edificio Emprendia, Campus Sur, 15782, Santiago de Compostela, Spain.

Technical support: email: [info@diversatechnologies.com](mailto:info@diversatechnologies.com) | [www.diversatechnologies.com](http://www.diversatechnologies.com)

**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 ANIONIC PEPTIDE DELIVERY NANOPARTICLES** protocol.

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

**DIVERSA TECHNOLOGIES S.L.** | Edificio Emprendia, Campus Sur, 15782, Santiago de Compostela, Spain.

Technical support: email: [info@diversatechnologies.com](mailto:info@diversatechnologies.com) | [www.diversatechnologies.com](http://www.diversatechnologies.com)

**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 *TITYUS STIGMURUS* ANIONIC PEPTIDE (TanP) ASSOCIATION PROTOCOL

---

1. Reconstitute the **DIV042F1** vial with 100  $\mu$ L 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  $\mu$ L of ultrapure water into the **DIVTECH** vial.
3. Add the content of **DIV042F1** to the **DIVTECH** vial using a micropipette and the provide narrow 1 mL micropipette tip.

**IMPORTANT:** Before adding the lipids from **DIV042F1** vial to **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** is now ready for the association of the TanP peptide. Alternatively, keep it at 4 °C and use it in the following 60 days.

4. Add 20  $\mu$ L of the **DIVERSA NANOPARTICLES** dropwise into 5  $\mu$ L of TanP solution and pipette up and down gently.  
**Note:** we recommend using peptide stock concentration at 4 mg/mL to increase the reproducibility.
5. Incubate the mixture for 15 min at RT.

The **DIVERSA FLUOGREEN PEPTIDE NANOPARTICLES** is now ready-to-use. Alternatively, keep it at 4 °C and use it in the following 2 days.

---

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

**DIVERSA TECHNOLOGIES S.L.** | Edificio Emprendia, Campus Sur, 15782, Santiago de Compostela, Spain.

Technical support: email: [info@diversatechnologies.com](mailto:info@diversatechnologies.com) | [www.diversatechnologies.com](http://www.diversatechnologies.com)

**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. Prepare the **DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES** following the provided protocol.

3. Add 960  $\mu$ L of fresh cell cultured medium supplemented with 10% (v/v) of FBS and, if necessary, 1 % (v/v) of antibiotics.

4. Add the **DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES** to a final volume of 1 mL.

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

5. Incubate the cells for 2-4 hours at 37 °C.

**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 ANIONIC PEPTIDE DELIVERY NANOPARTICLES** and, carefully, wash the cells twice with PBS 1X buffer and remove it.

The **DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY 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.

---

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

**DIVERSA TECHNOLOGIES S.L.** | Edificio Emprendia, Campus Sur, 15782, Santiago de Compostela, Spain.

Technical support: email: [info@diversatechnologies.com](mailto:info@diversatechnologies.com) | [www.diversatechnologies.com](http://www.diversatechnologies.com)

**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.

## OPTIMIZATION GUIDELINES

---

It is highly recommended to optimize your conditions to get the best **DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES** performance. Optimize one parameter at a time.

The following parameters can be optimized:

- **Amount of DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES:** Start fixing the concentration and amount of your anionic peptide to be delivered, and then you may vary the quantity of the **DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES**.
- **Amount of anionic peptide to be delivered:** you may need to vary the amount of your anionic peptide to be delivered as we recommended in [Table 2](#) (Recommendations of Use and Technical Notes). Depending on the sensitivity of your assay, a greater amount of peptide and **DIVERSA FLUOGREEN ANIONIC PEPTIDE DELIVERY NANOPARTICLES** may be required. For higher amounts of peptide, you can scale up the volume of **DIVERSA NANOPARTICLES** according to the amount of peptide.
- **Concentration of the peptide solution:** We recommend peptide concentration at 4 mg/mL. At lower concentrations, we recommend concentrating your peptide using ultracentrifugation filters 0.5 mL- 10 kDa. If your protein is in powder, we recommend dissolve it at final concentration of 4 mg/mL in its corresponding buffer.
- **Cell type and density:** you may need to optimize cell numbers. Delivery efficacy may be sensitive to the confluency of the cells in culture.
- **Incubation times for *in vitro* assays:** you may vary incubation times, depending on the type of functional assay performed, shorter or longer incubation time may influence delivery efficiency.

---

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

**DIVERSA TECHNOLOGIES S.L.** | Edificio Emprendia, Campus Sur, 15782, Santiago de Compostela, Spain.

Technical support: email: [info@diversatechnologies.com](mailto:info@diversatechnologies.com) | [www.diversatechnologies.com](http://www.diversatechnologies.com)

**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.



## RECOMMENDATIONS OF USE AND TECHNICAL NOTES

Table 1. Suggested aqueous solutions for **DIVTECH** vial.

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

Table 2. Suggested volumes for **DIVERSA FLUOGREEN PEPTIDE NANOPARTICLES**.

| DIVERSA NANOPARTICLES | PEPTIDE solution | Amount of PEPTIDE* |
|-----------------------|------------------|--------------------|
| 50 $\mu$ L            | 5 $\mu$ L        | 20-50 $\mu$ g      |
| 20 $\mu$ L            | 5 $\mu$ L        | 10-20 $\mu$ g      |
| 10 $\mu$ L            | 5 $\mu$ L        | 1-10 $\mu$ g       |

\*For higher amounts of peptide, you can scale up the volume of **DIVERSA NANOPARTICLES** according to the amount of peptide (e.g., 200  $\mu$ L of formulation for up to 200  $\mu$ g of peptide, ideally in 5  $\mu$ L, however, this peptide solution volume can be increased for higher quantities).

Table 3. Recommended volumes for cell culture.

| Cell culture vessel | Volume of DIVERSA | Volume of medium | Final volume/well |
|---------------------|-------------------|------------------|-------------------|
| 100 cm              | 200 $\mu$ L       | 4,8 mL           | 5 mL              |
| 6-well              | 40 $\mu$ L        | 960 $\mu$ L      | 1 mL              |
| 12-well             | 20 $\mu$ L        | 996 $\mu$ L      | 500 $\mu$ L       |
| 24-well             | 10 $\mu$ L        | 240 $\mu$ L      | 250 $\mu$ L       |
| 96-well             | 4 $\mu$ L         | 96 $\mu$ L       | 100 $\mu$ L       |

\*In 6-well plates,  $2-3 \times 10^5$  cells must be seeded per well. **Note:** the cell density should be optimized for each cell model.

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

**DIVERSA TECHNOLOGIES S.L.** | Edificio Emprendia, Campus Sur, 15782, Santiago de Compostela, Spain.

Technical support: email: [info@diversatechnologies.com](mailto:info@diversatechnologies.com) | [www.diversatechnologies.com](http://www.diversatechnologies.com)

**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 <b>DIV042F1</b> ?                                    | The concentration of the fluorophore is 4 µg/mL in the final <b>DIVERSA FLUOGREEN NANOPARTICLES</b> .   |
| Can I filter the formulation?  | Yes, if necessary, <b>DIVERSA FLUOGREEN</b> can be filtered using 0.22 µm filters of PES membrane.  |
| 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 20 µL of <b>DIVERSA FLUOGREEN-PEPTIDE NANOPARTICLES</b> with 180 µL of MilliQ water.   |
| Can I use <b>DIVERSA FLUOGREEN-PEPTIDE NANOPARTICLES</b> for research <i>in vivo</i> studies?        | Yes, <b>DIVERSA FLUOGREEN-PEPTIDE</b> can be used for research <i>in vivo</i> studies. For specific recommendations and a customized and optimized prototype, contact <b>DIVERSA</b> .  |
| What if I need to work with higher protein/peptide concentrations than the ones provided in Table 2? | You can concentrate the formulation (see next question), or alternatively, contact <b>DIVERSA</b> for advice depending on your specific protein/peptide.  |
| How do I concentrate the formulation?  | If necessary, the 1 mL of <b>DIVERSA FLUOGREEN-PEPTIDE NANOPARTICLES</b> can be concentrated by using a SpeedVac or Rotavap in mild conditions (avoid surpassing 35 °C or drying the samples). Samples can be concentrated up to 4-fold its original volume (i.e., to a final volume 250 µL). |

## ONLINE RESOURCES

Visit our website [www.diversatechnologies.com](http://www.diversatechnologies.com) for further information.

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

**DIVERSA TECHNOLOGIES S.L.** | Edificio Emprendia, Campus Sur, 15782, Santiago de Compostela, Spain.

Technical support: email: [info@diversatechnologies.com](mailto:info@diversatechnologies.com) | [www.diversatechnologies.com](http://www.diversatechnologies.com)

**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.