

DIVERSA ANIONIC PEPTIDE DELIVERY NANOPARTICLES

Enhancing intracellular delivery of anionic peptides

USER PROTOCOL – #DIV042

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ABOUT THE NANOPARTICLES

OVERVIEW

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

DIVERSA 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.

COMPONENTS

- 1x **DIV042** vial for reconstitution.
- 1x **DIVTECH** vial for preparation of **DIVERSA PEPTIDE** nanoparticles.
- 2x Tips for 1 mL micropipette.

STORAGE

Before formulating, store the vials at $-20\text{ }^{\circ}\text{C}$. Once formulated, the preparation should be stored at $2\text{--}8\text{ }^{\circ}\text{C}$ for up to 60 days without the peptide, or up to 2 days with the associated peptide.

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: support@diversatechnologies.com | <https://www.diversatechnologies.com>

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EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

- 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

- 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**'s nanoparticles.
- **DIVERSA** cannot guarantee the optimal formulation performance if any modifications are made to the protocol.
- It is recommended to use **DIVERSA**'s nanoparticles (prior association of the peptide) within 60 days of preparation for optimal performance.
- After anionic peptide association, it is recommended to use **DIVERSA PEPTIDE** nanoparticles within 48 hours for optimal performance.
- **DIVERSA 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-100, SDS or Tween-20 for the preparation of **DIVERSA PEPTIDE** nanoparticles.
- Once formulated, do NOT freeze **DIVERSA PEPTIDE** nanoparticles.
- Do NOT heat over 90 °C **DIVERSA PEPTIDE** nanoparticles.

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DIVERSA ANIONIC PEPTIDE DELIVERY NANOPARTICLES PROTOCOL

FORMULATION STEP

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

1. Add 100 μL of EtOH into the **DIV042** vial. Gently pipette up and down.
2. Add 900 μL of ultrapure water into the **DIVTECH** vial.
3. Transfer the entire volume from **DIV042** vial to the **DIVTECH** vial using a 1 mL micropipette and the provided tip.

Note: Before adding the volume from **DIV042** vial to 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**' 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.

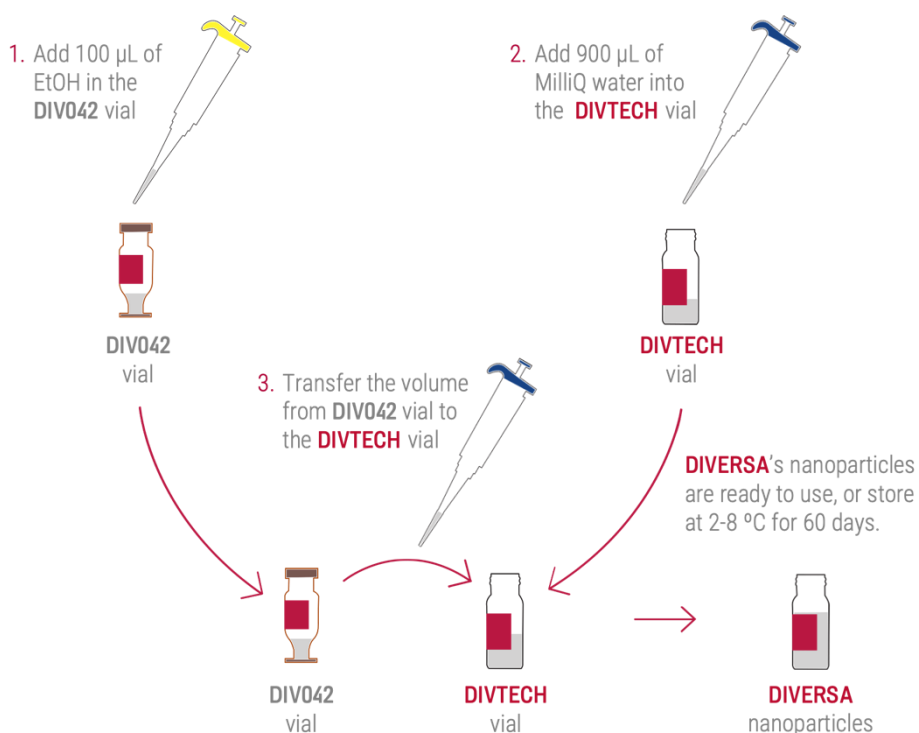


Figure 1. DIVERSA ANIONIC PEPTIDE DELIVERY NANOPARTICLES: FORMULATION STEP.

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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**' 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**' nanoparticles to the **PEPTIDE** solution. Pipette up and down 5-10 times with confidence.
3. Incubate the **DIVERSA PEPTIDE** nanoparticles at room temperature (RT) for 15 min. Agitation is not required.

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

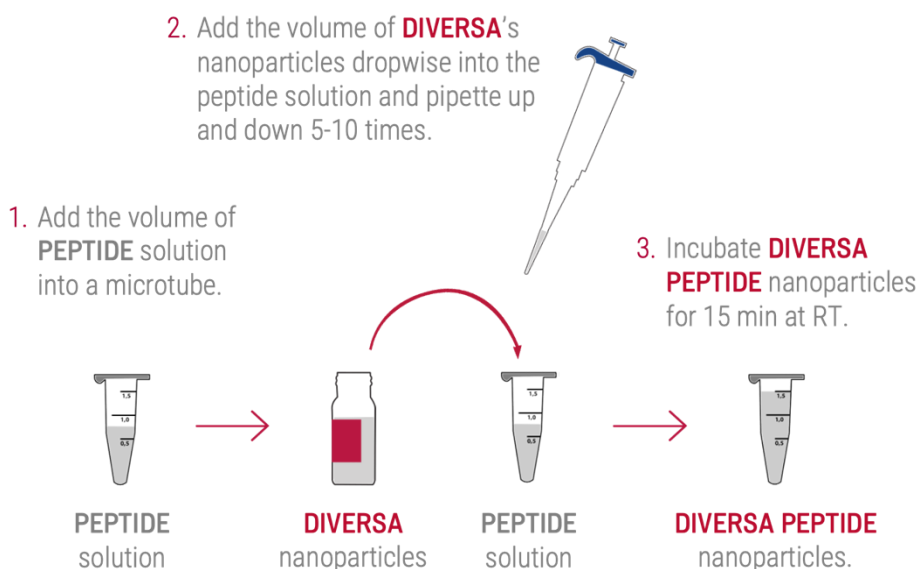


Figure 2. DIVERSA ANIONIC PEPTIDE DELIVERY NANOPARTICLES: PEPTIDE ASSOCIATION STEP.

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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 µL 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's** 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 µg (i.e., 25 µL) and dilute it up to 40 µL with MilliQ water. Then, add the 60 µL of **DIVERSA's** nanoparticles dropwise onto the **PEPTIDE** solution following the steps 1-3 of the PEPTIDE ASSOCIATION STEP. The **DIVERSA L-Glutathione** nanoparticles are ready to use or can be stored at 2-8 °C for up to 2 days.
4. Carefully remove the culture medium from each well. Add 900 µL of fresh complete medium, then add 100 µL of the **DIVERSA L-Glutathione** nanoparticles prepared in Step 3 (containing 25 µg of peptide), bringing the final volume to 1 mL per well.
5. Incubate the cells at 37 °C in a CO₂ 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.

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

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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 PEPTIDE** nanoparticles

Amount of ANIONIC PEPTIDE (µg)	Volume of Peptide solution (µL)	Volume of DIVERSA's 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**'s 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 of **DIVERSA PEPTIDE** nanoparticles to add to cell culture medium depending on the cell culture vessel.

Cell culture vessel	Volume of DIVERSA 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
Can I filter the formulation?	Yes, DIVERSA PEPTIDE 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 (DLS) analysis.
Can I use DIVERSA PEPTIDE nanoparticles for in vivo studies?	Yes, DIVERSA PEPTIDE nanoparticles can be used <i>in vivo</i> . DIVERSA 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 DIVERSA .
How do I concentrate the formulation?	DIVERSA PEPTIDE 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

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

CHANGELOG

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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