

DIVTECH FLUOGREEN ANIONIC PROTEIN/PEPTIDE KIT

Tracking intracellular delivery of anionic proteins/peptides

USER PROTOCOL – #DIV042F1

ABOUT THE KIT	1
OVERVIEW	1
COMPONENTS	1
STORAGE.....	1
CONSIDERATIONS BEFORE STARTING.....	2
DIVTECH FLUOGREEN ANIONIC PROTEIN/PEPTIDE KIT	3
PROTOCOL	3
EXAMPLE OF ITGa6 β 4 ASSOCIATION PROTOCOL.....	4
RECOMMENDATIONS OF USE AND TECHNICAL NOTES	5
FREQUENTLY ASKED QUESTIONS.....	6
ONLINE RESOURCES	6

ABOUT THE KIT

OVERVIEW

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

DIVTECH FUOGREEN ANIONIC PROTEIN/PEPTIDE KIT, based on cationic lipids, is suitable for an efficient association of your anionic proteins/peptides ($\text{pH} > \text{pI}$) mainly due to electrostatic interactions.

COMPONENTS

- 1x **DIV042F1** vial for reconstitution.
- 1x **DIVTECH** vial for preparation of **DIVTECH** formulation.
- 2x Tips for 1 mL micropipette.

STORAGE

Before formulating, store the vials at $-20\text{ }^{\circ}\text{C}$. Once formulated storage is recommended at $4\text{ }^{\circ}\text{C}$.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

- 1 mL micropipette.
- MilliQ water or any other recommended buffer.
- Ethanol 96%.
- DMSO.
- Protein/peptide of interest.

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

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Technical support: email: info@diversatechnologies.com | www.diversatechnologies.com

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CONSIDERATIONS BEFORE STARTING

- The following protocol is optimized for the preparation of 1 mL of **DIVTECH FLUOGREEN** formulation (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 **DIVTECH FLUOGREEN** formulation within 60 days.
- **DIVTECH FLUOGREEN** formulation is stable for 24 h in cell culture media at 37 °C: DMEM, RPMI (with/without FBS).
- Do NOT use any buffer solution containing Triton-X, SDS or Tween 20 for the preparation or manipulation of **DIVTECH FLUOGREEN/ DIVTECH FLUOGREEN-PROTEIN/PEPTIDE** formulation.
- Do NOT freeze **DIVTECH FLUOGREEN / DIVTECH FLUOGREEN-PROTEIN/PEPTIDE** formulation.
- Do NOT heat up **DIVTECH** at temperatures higher than 90 °C.
- Do NOT centrifuge or vortex **DIVTECH FLUOGREEN / DIVTECH FLUOGREEN-PROTEIN/PEPTIDE** formulation.

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DIVTECH FLUOGREEN ANIONIC PROTEIN/PEPTIDE KIT PROTOCOL

1. Reconstitute the **DIV042F1** vial with 100 μ L of EtOH and gently pipette up and down for mixing.
2. Add 900 μ L of ultrapure water (milliQ) into the **DIVTECH** vial or, alternatively, a buffer solution suggested in [Table 1](#) (Recommendations of Use and Technical Notes).
3. Add the content of **DIV042F2** to the **DIVTECH** vial, using a **1 mL micropipette**, in order to have more air volume for mixing in a fast and vigorous way.

The **DIVTECH** formulation is now ready for the association of the protein/peptide. Alternatively, keep it at 4 °C and use it in the following 60 days.

4. Add the **DIVTECH** formulation gently and dropwise into the protein/peptide solution. Recommended volumes are provided in [Table 2](#).
5. Incubate the mixture for 15 min at room temperature.

The **DIVTECH-PROTEIN/PEPTIDE** formulation is now ready-to-use. Alternatively, keep it at 4 °C and use it in the following 2 days.

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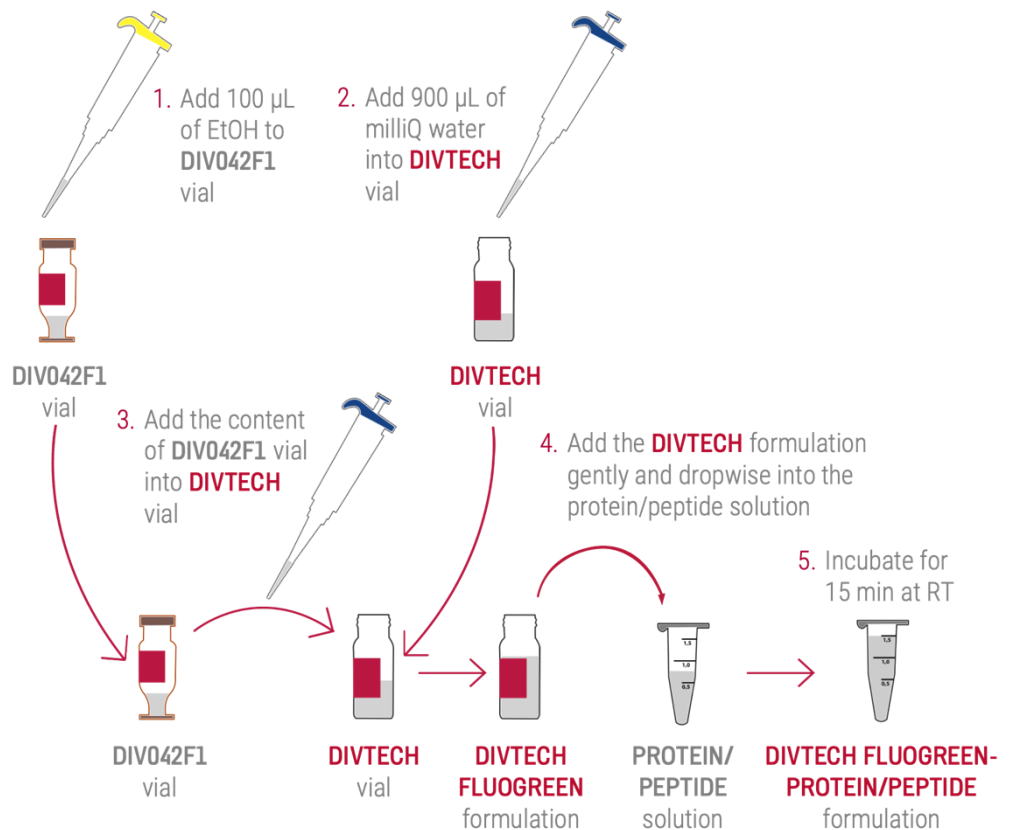


Figure 1. DIVTECH FLUOGREEN ANIONIC PROTEIN/PEPTIDE Kit protocol.

EXAMPLE OF ITGa6β4 ASSOCIATION PROTOCOL

1. Reconstitute the **DIV042** vial with 100 µL of EtOH and gently pipette up and down for mixing.
2. Add 900 µL of ultrapure water (milliQ) into the **DIVTECH** vial
3. Add the content of **DIV042** to the **DIVTECH** vial.
4. Take 10 µL the **DIVTECH** formulation and add gently and dropwise into 5 µL of ITGa6β4 (MW = 188.8 kDa) protein solution (2 mg/mL).
5. Incubate the mixture for 15 min at room temperature.

The **DIVTECH-PROTEIN** formulation is now ready-to-use. Alternatively, keep it at 4 °C and use it in the following 2 days.

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RECOMMENDATIONS OF USE AND TECHNICAL NOTES

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

AQUEOUS SOLUTION	CONCENTRATION
Ultrapure water	N/A
NaCl	150 mM
HEPES	10-25 mM

Table 2. Suggested volumes for **DIVTECH-PROTEIN/PEPTIDE** formulation.

DIVTECH formulation	PROTEIN/PEPTIDE solution	Amount of PROTEIN/PEPTIDE
50 µL	5 µL	20-50 µg
20 µL	5 µL	10-20 µg
10 µL	5 µL	1-10 µg

Table 3. 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 µL	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

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FREQUENTLY ASKED QUESTIONS

QUESTION	ANSWER
What is the concentration of the fluorophore in DIV042F1?	The concentration of the fluorophore is 4 µg/mL in the final DIVTECH FLUOGREEN formulation.
Can I filter the formulation?	Yes, if necessary, DIVTECH FLUOGREEN can be filtered using 0.22 µm filters
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 DIVTECH FLUOGREEN-PROTEIN/PEPTIDE formulation with 180 µL of milliQ water.
Can I use DIVTECH FLUOGREEN-PROTEIN/PEPTIDE formulation for research <i>in vivo</i> studies?	Yes, DIVTECH FLUOGREEN-PROTEIN/PEPTIDE can be used for research <i>in vivo</i> studies. For specific recommendations and a customized and optimized prototype, contact DIVERSA .
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 DIVERSA for advice depending on your specific protein/peptide.
How do I concentrate the formulation?	If necessary, the 1 mL of DIVTECH FLUOGREEN-PROTEIN/PEPTIDE formulation 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 for further information.

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