

## **DIVTECH FLUOGREEN UPTAKE KIT**

Tracking fluorescent DIVTECH for an effective cell internalization

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### **USER PROTOCOL – #DIV000F1**

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## ABOUT THE KIT

### OVERVIEW

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**DIVTECH** is a biocompatible, biodegradable, and cell-friendly technology for enhancing intracellular delivery of small molecules and biomolecules, paving the way towards clinical translation.

**DIVTECH FLUOGREEN UPTAKE KIT** uses strongly labelled fluorescent nanometric emulsions that are easily internalized by live cells. They can be visualized in less than two hours at Ex / Em (495/503 nm) with a wide variety of platforms: flow cytometry, microplate assays, fluorescence, and confocal microscopy.

**DIVTECH FLUOGREEN** formulation can be used as a positive control for cell internalization before testing the efficiency of associated molecules in specific cell lines of interest.

**DIVTECH FLUOGREEN** formulation 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

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- 1x **DIV000F1** vial for reconstitution.
- 1x **DIVTECH** vial for preparation of each **DIVTECH FLUOGREEN** formulation.
- 2x Tips for 1 mL micropipette.

### STORAGE

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

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

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## EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

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- 1 mL micropipette
- MilliQ water or any other recommended buffer
- Ethanol 96%

## CONSIDERATIONS BEFORE STARTING

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- The following protocol is optimized for the preparation of 1 mL of **DIVTECH FLUOGREEN** formulation (starting from one **DIV000F1** 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 the following 24-48 h.
- **DIVTECH FLUOGREEN** formulation is stable for 24 h in cell culture media at 37 °C: DMEM and 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** formulation.
- Do NOT freeze **DIVTECH FLUOGREEN** formulation.
- Do NOT heat up **DIVTECH FLUOGREEN** formulation at temperatures higher than 90 °C for more than 2 h.
- Do NOT centrifuge or vortex **DIVTECH FLUOGREEN** formulation.

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## DIVTECH FLUOGREEN UPTAKE KIT PROTOCOL

1. Reconstitute the **DIV000F1** vial with 100  $\mu\text{L}$  of EtOH and gently pipette up and down for mixing.
2. Add 900  $\mu\text{L}$  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 **DIV000F1** to the **DIVTECH** vial, using a 1 mL micropipette and the provided narrow 1 mL micropipette tip, in order to have more air volume for mixing in a fast and vigorous way.

The **DIVTECH FLUOGREEN** formulation is spontaneously formed and ready-to-use ([Table 2](#)). Alternatively, keep it at 4 °C and use it in the following 24-48 h.

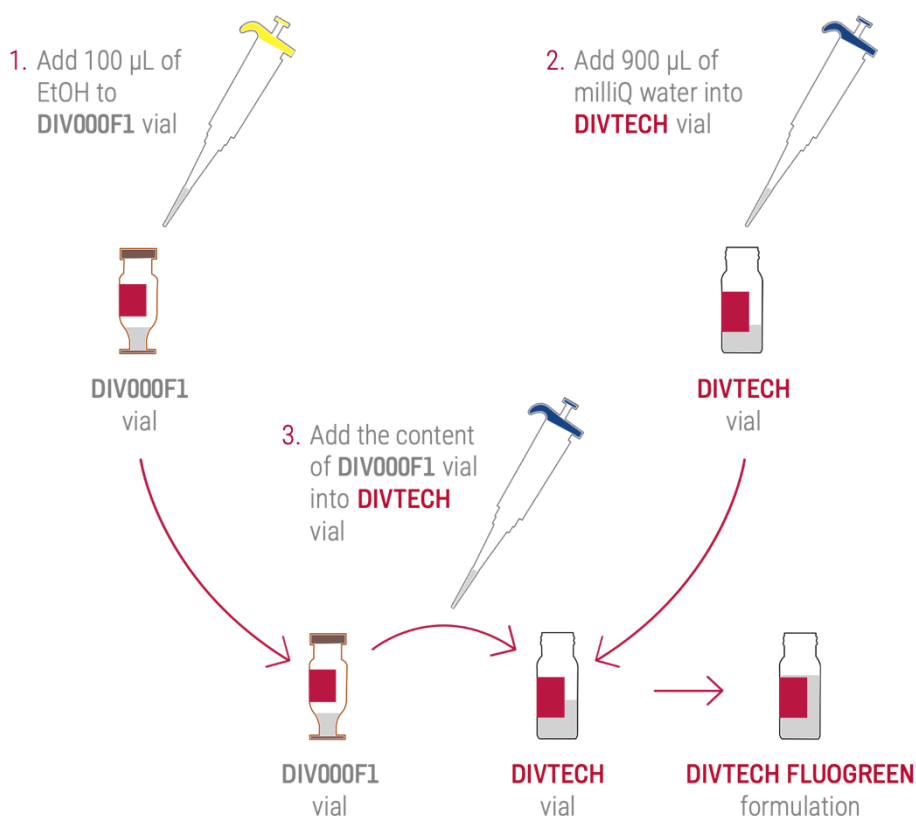


Figure 1. **DIVTECH FLUOGREEN UPTAKE KIT** protocol.

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## EXAMPLE OF UPTAKE PROTOCOL

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1. Seed 500.000 A549 cells / well in a 6-well plate the day before the uptake experiment for FACS analysis.
2. Reconstitute the **DIV000F1** vial with 100 µL of EtOH and gently pipette up and down for mixing.
3. Add 900 µL of ultrapure water (milliQ) into the **DIVTECH** vial.
4. Add the content of the **DIV000F1** vial to the **DIVTECH** vial.
5. Add 960 µL of fresh complete medium to each well.
6. Add 40 µL of **DIVTECH FLUOGREEN** to each well.
7. Incubate the cells at 37 °C in a CO2 incubator under standard conditions for 2-4 hours.
8. Harvest, wash the cells and adjust the concentration for analysis on the flow cytometer (Ex/Em = 495/503 nm).

Note: We recommend analysis on the same day. For extended storage (>16 h) as well as for greater flexibility in planning on the cytometer, resuspend cells in 1-4% paraformaldehyde to prevent deterioration.

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## RECOMMENDATIONS OF USE & TECHNICAL NOTES

Table 1. Suggested buffers for **DIVTECH** vial.

AQUEOUS SOLUTION	CONCENTRATION
Ultrapure water	N/A
PBS	2-50 mM
NaCl	150 mM
HEPES	10-25 mM

Table 2. Recommended volumes for cell culture.

Cell culture vessel	Volume of <b>DIVTECH</b>	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

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## FREQUENTLY ASKED QUESTIONS

QUESTION	ANSWER
What is the concentration of the fluorophore in DIV000F1?	The concentration of the fluorophore is 4 µg/mL in the final <b>DIVTECH FLUOGREEN</b> formulation.
How do I concentrate the formulation?	If necessary, the 1 mL of <b>DIVTECH FLUOGREEN</b> formulation can be concentrated by using a SpeedVac or Rotavap in mild conditions (avoid surpassing 35 °C or drying out the samples). Samples can be concentrated up to 4-fold its original volume (i.e., to a final volume 250 µL).
Can I filter the formulation?	Yes, if necessary, <b>DIVTECH FLUOGREEN</b> formulation can be filtered using 0.22 µm filters.
Can I use buffers other than milliQ water?	Yes, please check Table 1 for other recommended buffers.
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 10 µL of <b>DIVTECH FLUOGREEN</b> formulation with 90 µL of milliQ water.
Can I use <b>DIVTECH FLUOGREEN</b> formulation for <i>in vivo</i> studies?	Yes, <b>DIVTECH FLUOGREEN</b> formulation can be used <i>in vivo</i> . For specific recommendations and a customized and optimized prototype, contact <a href="#">DIVERSA</a> .

## ONLINE RESOURCES

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

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