

DIVTECH FLUOGREEN SMALL MOLECULE KIT

Tracking intracellular delivery of small molecules

USER PROTOCOL - #DIV010F1

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ABOUT THE KIT

OVERVIEW

DIVTECH is a biocompatible, biodegradable, and cell-friendly technology for enhancing intracellular delivery of hydrophobic drugs, paving the way towards clinical translation.

DIVTECH FLUOGREEN SMALL MOLECULE KIT 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 two hours at Ex/Em = 495/503 nm.

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.

COMPONENTS

- 1x **DIV010F1** vial for reconstitution.
- 1x **DIVTECH** vial for preparation of each **DIVTECH FLUOGREEN-DRUG** formulation.
- 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 | www.diversatechnologies.com

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EQUIPMENT AND MATERIALS REQUIRED BUT NOT SUPPLIED

- 1 mL micropipette.
- MilliQ water or any other recommended buffer.
- Ethanol 96%.
- Drug of interest.

CONSIDERATIONS BEFORE STARTING

- The following protocol is optimized for the preparation of 1 mL of **DIVTECH FLUOGREEN-DRUG** formulation (starting from one **DIV010F1** 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-DRUG** formulation within 24-48 h.
- **DIVTECH FLUOGREEN-DRUG** 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-DRUG** formulation.
- Do NOT freeze **DIVTECH FLUOGREEN-DRUG** formulation.
- Do NOT heat up **DIVTECH FLUOGREEN-DRUG** formulation at temperatures higher than 90 °C for more than 2 h.
- Do NOT centrifuge or vortex **DIVTECH FLUOGREEN-DRUG** formulation.

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DIVTECH FLUOGREEN SMALL MOLECULE KIT PROTOCOL

1. Reconstitute the **DIV010F1** vial with 20 μL of EtOH and gently pipette up and down for mixing.
2. Add the drug dissolved in one of the solvents suggested in [Table 1](#) (Recommendations of Use and Technical Notes) to **DIV010F1** vial, considering the maximum volume for each solvent.
3. Adjust the volume of the mixture with EtOH up to a final volume of 100 μL .
4. Add 900 μL of ultrapure water (milliQ) into the **DIVTECH** vial or, alternatively, an aqueous solution suggested in [Table 2](#) (Recommendations of Use and Technical Notes).
5. Add the content of **DIV010F1** containing the drug 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-DRUG** formulation is spontaneously formed and now ready-to-use ([Table 3](#)). Alternatively, keep it at 4 °C and use it in the following 24-48 h.

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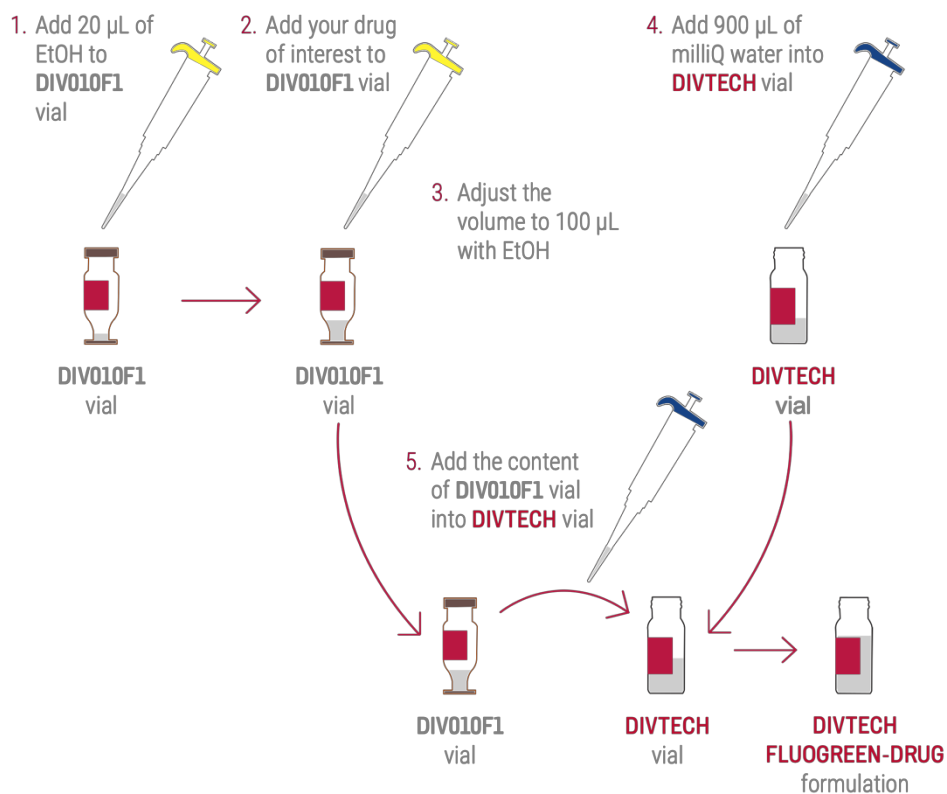


Figure 1. **DIVTECH FLUOGREEN SMALL MOLECULE KIT** protocol.

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RECOMMENDATIONS OF USE & TECHNICAL NOTES

Table 1. This table shows the maximum volumes of solvents in which the drugs can be dissolved and incorporated to the DIV010F1 vial.

SOLVENT*	VOLUME
EtOH	Up to 80 µL
DMSO	≤ 25 µL
MeOH	≤ 25 µL
ACN	≤ 25 µL
Acetone	≤ 25 µL
Chloroform	≤ 10 µL

*Solvent of preference: EtOH.

Note: Irrespective of the solvent, keep the drug concentration in the preparation (1 mL) lower than 75 mg/mL.

Table 2. Suggested buffers for DIVTECH vial.

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

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Table 3. Recommended volumes for cell culture.

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

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FREQUENTLY ASKED QUESTIONS

QUESTION	ANSWER
What is the concentration of the fluorophore in DIV010F1?	The concentration of the fluorophore is 4 µg/mL in the final DIVTECH FLUOGREEN formulation.
How do I concentrate the formulation?	If necessary, the 1 mL of DIVTECH FLUOGREEN-DRUG formulation can be concentrated by using a SpeedVac or Rotavap in mild conditions (avoid overpassing 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, DIVTECH FLUOGREEN-DRUG formulation can be filtered using 0.22 µm filters.
What should I do if my drug is not soluble in EtOH?	You can use any solvent listed in Table 1 . You can also sonicate DIV010F1 vial containing the drug prior addition to the DIVTECH vial.
Can I use buffers other than milliQ water?	Yes, please check Table 2 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 DIVTECH FLUOGREEN-DRUG formulation with 90 µl of milliQ water.
Can I use DIVTECH for <i>in vivo</i> studies?	Yes, DIVTECH FLUOGREEN-DRUG can be used <i>in vivo</i> . For specific recommendations and a customized and optimized prototype, contact DIVERSA .

ONLINE RESOURCES

Visit our website www.diversatechnologies.com for further information.

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