

DIVTECH SMALL MOLECULE KIT

Enhancing intracellular delivery of small molecules

USER PROTOCOL - #DIV010

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ABOUT THE KIT

OVERVIEW

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

DIVTECH SMALL MOLECULE KIT is suitable for hydrophilic and hydrophobic small molecules and can be used to efficiently deliver your compound/drug of interest to specific cell lines. **DIVTECH** prevents loaded drugs from external degradation and rapid clearance. It promotes optimal and targeted biodistribution, high intracellular absorption and low toxicity, boosting their therapeutic effect.

COMPONENTS

- 4x **DIV010** vials for reconstitution.
- 4x **DIVTECH** vials for preparation of each **DIVTECH-DRUG** formulation.
- 8x 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-DRUG** formulation (starting from one **DIV010** 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-DRUG** formulation within 24-48 h.
- **DIVTECH-DRUG** formulation is stable for 6 h in cell culture media at 37 °C: DMEM and RPMI (with/without FBS).
- Do NOT use any buffer solution containing Triton X-100, SDS or Tween 20 for the preparation or manipulation of **DIVTECH-DRUG** formulation.
- Do NOT freeze **DIVTECH-DRUG** formulation.
- Do NOT heat up **DIVTECH-DRUG** formulation at temperatures higher than 90 °C for more than 2 h.
- Do NOT centrifuge or vortex **DIVTECH-DRUG** formulation.

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DIVTECH SMALL MOLECULE KIT PROTOCOL

1. Reconstitute the **DIV010** 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 **DIV010** 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 **DIV010** 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-DRUG** formulation is spontaneously formed and ready-to-use. Alternatively, keep it at 4 °C and use it in the following 24-48 h.

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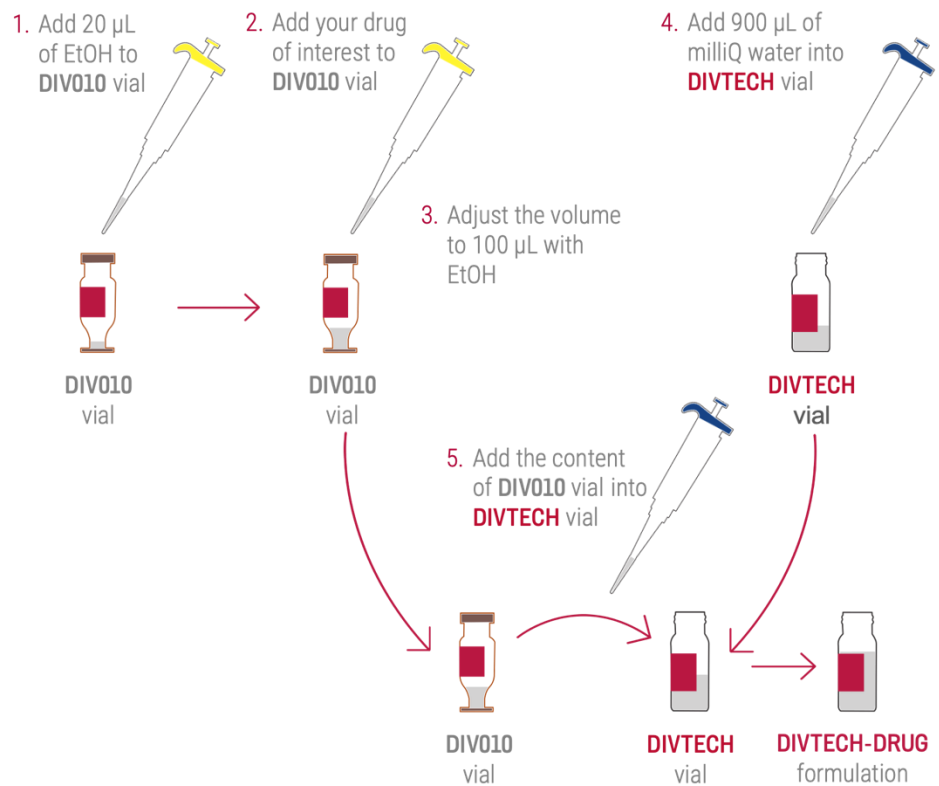


Figure 1. **DIVTECH Small Molecule Kit** protocol.

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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 DIV010 vial.

SOLVENT*	VOLUME
EtOH	Up to 80 μ L
DMSO	\leq 25 μ L
MeOH	\leq 25 μ L
ACN	\leq 25 μ L
Acetone	\leq 25 μ L
Chloroform	\leq 10 μ L

*Solvent of preference: EtOH.

Note: Irrespective of the solvent, keep the drug concentration in the preparation (1 mL) lower than 0.75 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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FREQUENTLY ASKED QUESTIONS

QUESTION	ANSWER
How do I concentrate the formulation?	If necessary, the 1 mL of DIVTECH-DRUG 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, DIVTECH-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 DIV010 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-DRUG formulation with 90 µL of milliQ water.
Can I use DIVTECH for <i>in vivo</i> studies?	Yes, DIVTECH 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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