DIVERSA

DIVERSA Technologies Application Note

Discover all the technical features of the new DIVTECH Lipid nanoemulsion delivery system.

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Facilitators of Life Sciences Research

PHYSICAL CHARACTERIZATION

DIVTECH are ready-to-use formulations to obtain encapsulating nanosystems in a fast and easy way. **DIVTECH** show an easily controllable size, narrow distribution, high reproducibility, neutral surface charge, and a spherical shape (Fig. 1).

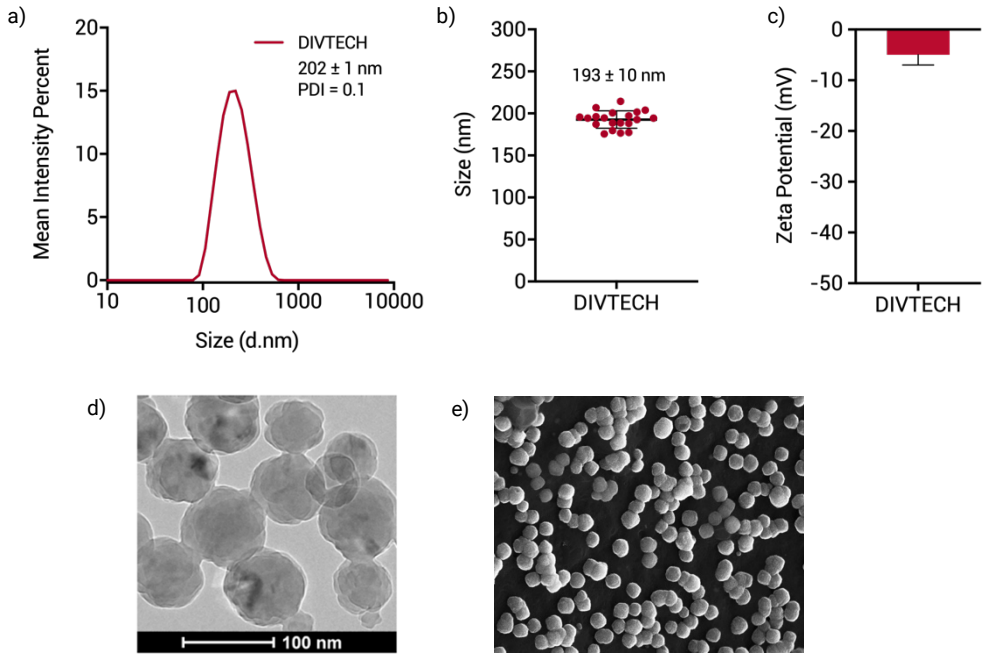


Figure 1. Characterization of **DIVTECH** by the diameter size using Dynamic Light Scattering (DLS) (a), reproducibility of different batches (b) and Zeta Potential (c). Morphology observed by Cryogenic Transmission Electron Microscopy, Cryo-TEM (d) and Scanning Transmission Electron Microscopy (STEM) of **DIVTECH** (e).

STABILITY

In addition, **DIVTECH** has great stability in different relevant biological fluids (Table 1), during storage up to 6 months (Fig. 2a), in different buffers, and in culture media supplemented with FBS (Fig. 2b). Importantly, **DIVTECH** with the associated biomolecules (peptides and proteins) and small molecules (Table 2), maintains its stability (Fig. 3).

Table 1. Stability of the blank DIVTECH in different relevant fluids.

	Human plasma	Simulated tear fluid	Simulated synovial fluid	Simulated gastric fluid	Simulated intestinal fluid (SIF)	Fed state SIF	Fasted state SIF
DIVTECH	24 h*	24 h*	24 h*	6 h	4 h	4 h	24 h*

* End of the experiment, stable particles.

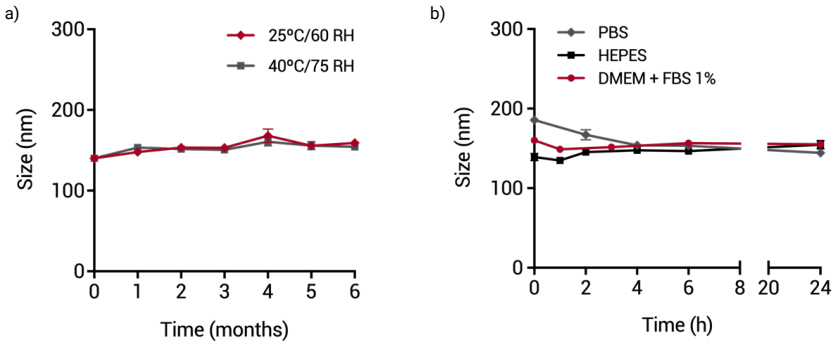


Figure 2. ICH (International Council for Harmonization) long-term stability of the blank **DIVTECH** at room temperature (40 C, 75% RH) and under storage conditions (25 C, 60% RH) (a). Stability of the blank **DIVTECH** in different buffers and cell culture medium supplemented with FBS (b).

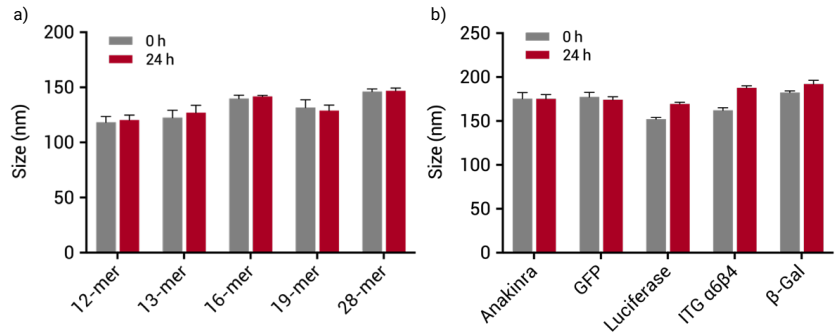


Figure 3. Stability of **DIVTECH** associated to peptides of different length (from 12 to 28 amino acids) (a) and associated to proteins of different MW, isoelectric point, and log P_{OW} (b) for 24 hours in storage conditions. Data represent the hydrodynamic diameter in nanometers (mean SD).

Table 2. Stability of **DIVTECH** associated to small molecules.

DIVTECH: Small molecule	Stability
Galunisertib	7 days*
Etoposide	15 days*
Paclitaxel	15 days*
Doramapimod	7 days*
Doxorubicin	15 days*

* End of the experiment, stable particles.

TOXICITY

DIVTECH showed a great biocompatibility with different cell types as well as *in vivo* models such as mice and zebrafish (Fig. 4).

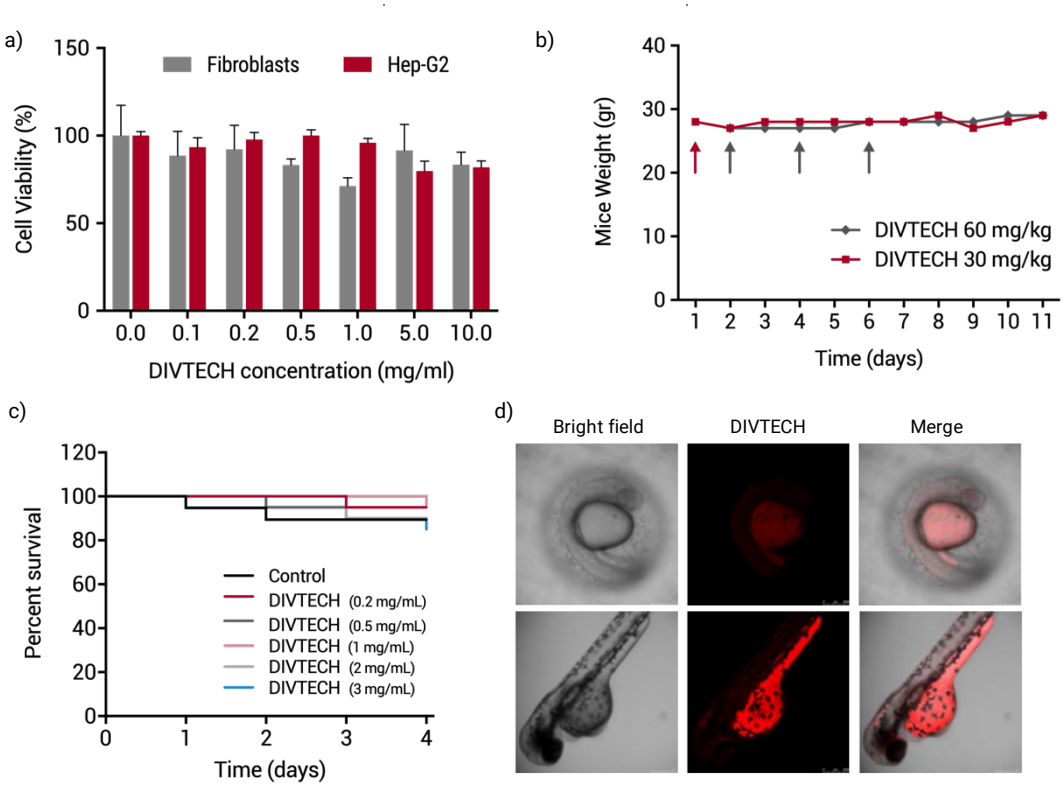


Figure 4. Evaluation of cytotoxicity effects of the blank **DIVTECH** after 48h incubation in hepatic cancer cell line HepG2 and fibroblasts. Data shown represent the mean values and S.E.M obtained in a triplicate (a). *In vivo* toxicity evaluation of the blank **DIVTECH** based on the body weight of healthy mice (n=6 mice each group). Red arrow: one dose tail intravenous injection of 30 mg/kg (10 mg/mL nanosystem concentration). Grey arrows: Three consecutive dose tail intravenous injection of 60 mg/kg (20 mg/mL nanosystem concentration) at days 2, 4 and 6 (cumulative dose 180mg/kg). No toxicity was observed in any of the cases (b). *In vivo* toxicity evaluation of the blank **DIVTECH** at different concentrations (from 0.2 to 3 mg/mL) in zebrafish embryos with chorionic membrane (n = 60 embryos in total) (c). Internalization of Nile-red labelled **DIVTECH** in zebrafish embryo model with and without the chorionic membrane (0.5 mg/ml) (d).

ASSOCIATION OF THERAPEUTIC MOLECULES

DIVTECH can associate a wide variety of therapeutic compounds as hydrophobic or amphiphilic molecules, peptides, proteins, and oligonucleotides (such as DNA and RNA, among others) showing great association efficiencies (table 3) and maintaining the nanometric size (Fig. 5). The specifications of some of the molecules that were efficiently associated with **DIVTECH** are detailed in tables 4 7.

Table 3. Association of different therapeutic molecules to **DIVTECH**.

Molecules	Association Efficiency (%AE)
Small molecules	85- 99 %
Peptides	70- 99 %
Proteins	70- 99 %
Oligonucleotides	85- 99 %

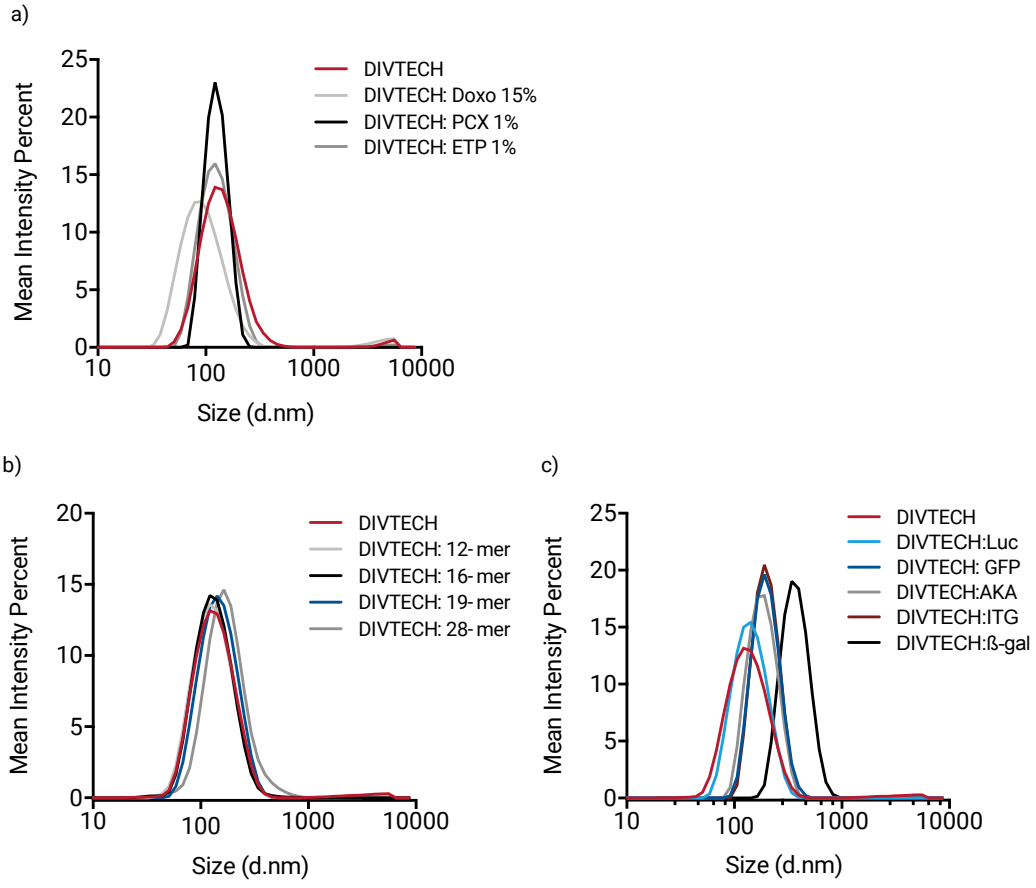


Figure 5. Characterization of **DIVTECH** by Dynamic Light Scattering (DLS) showing the hydrodynamic diameter of **DIVTECH** associated to small molecules (a), peptides of different length (from 12 to 28 amino acids) (b) and associated to proteins of different MW, isoelectric point, and log P_{ow} (c).

Table 4. Association of different small molecules to **DIVTECH**.

Drug	MW (g/mol)	Log P_{ow}	Mass (mg)	% Loading (w/w)	Molar (mM)	% EE
Galunisertib	369.42	2.4	0.75	15%	2.03	>90%
Doramapimod	527.66	5.7	0.75	15%	1.42	>99%
Disulfiram	296.54	3.9	0.75	15%	2.53	>90%
Paclitaxel	853.91	2.5	0.05	1%	0.06	>80%
Etoposide	588.56	0.6	0.05	1%	0.09	>80%
Doxorubicin	543.52	1.3	1	20%	1.84	>90%
Oleuropein	540.51	-0.4	0.75	15%	1.39	>99%
Rose Bengal	973.67	8.5	1	20%	1.03	>95%

Table 5. Comparative characterization of different commercially available competitors associating small molecules.

Drug (Loading)	Competitors	Size (nm)	PdI	Encapsulation Efficiency (%EE)
Galunisertib (15%)	Liposomes 1	111	0.26	< 40%
	Liposomes 2	1325	0.64	Precipitated
	Niosomes	940	0.56	Precipitated
	DIVTECH	**129**	**0.08**	**> 95%**
Doramapimob (15%)	Liposomes 1	110	0.22	Precipitated
	Liposomes 2	377	0.95	Precipitated
	Niosomes	318	0.91	Precipitated
	DIVTECH	**150**	**0.06**	**> 99%**
Oleuropein (15%)	Liposomes 1	110	0.22	< 46%
	Liposomes 2	377	0.95	Precipitated
	Niosomes	318	0.91	Precipitated
	DIVTECH	**150**	**0.06**	**> 99%**

Table 6. Association of different peptides to **DIVTECH**.

Peptide length	pI	Peptide charge	% Hydrophobic Aa
3-mer	5.9	-1	0 %
12-mer	9.5	+4	25 %
13-mer	9.8	+5	23 %
16-mer	9.5	+7	50 %
19-mer	8.6	+2	21 %
28-mer	11.9	+6	39 %

Table 7. Association of different proteins to **DIVTECH**.

Protein	MW (kDa)	pI	DIV031 (BR)	DIV042 (Anionic)
Anakinra	17.3	5.5	√	√
Green Fluorescent Protein	28.7	~6.0	√	
Luciferase (LucR8)	37.0	8.3	√	
Ovalbumin	44.5	4.6	√	√
Integrin α6β4	188.8	5.5	√	√
R-Phycoerythrin	250.0	5.6	√	√
β-Galactosidase	540.0	4.6	√	√

INTERNALIZATION

DIVTECH can be internalized reaching great efficiencies in short times, and by different cell types (Fig. 6) to achieve intracellular delivery of therapeutic molecules, such as peptides, proteins, or oligonucleotides (Fig. 7, 8) not only *in vitro*, but also *in vivo* (Fig. 9). **DIVTECH** can be employed in different applications as: drug delivery and targeting, delivery of peptides, drug and proteins, cosmetics, functional foods, nutraceuticals, etc.

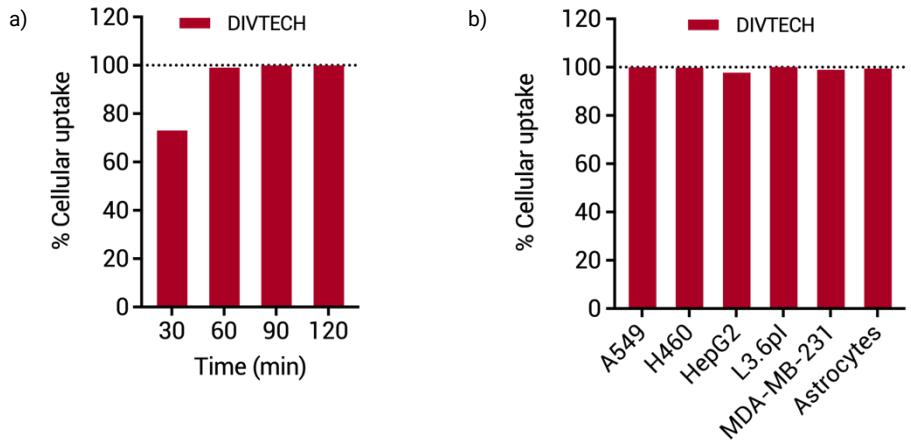


Figure 6. Time-depending uptake of fluorescent labeled **DIVTECH** by flow cytometry (a). Internalization of **DIVTECH** in different stablished cell lines and primary culture cells (b).

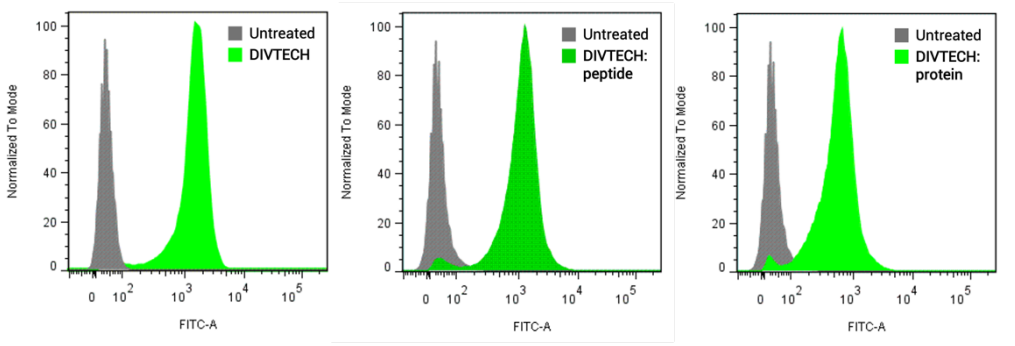
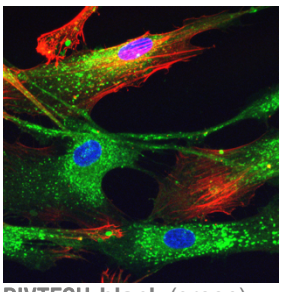


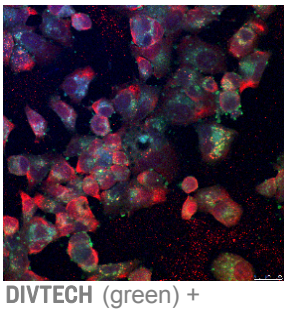
Figure 7. Uptake of fluorescent labeled **DIVTECH** by flow cytometry. Nearly 100% of cells become green after 2-hours incubation with **DIVTECH**. **DIVTECH** is efficiently internalized by cells with or without association of a peptide/protein.

MDA-MB-231



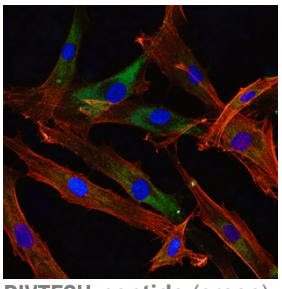
DIVTECH blank (green)

MCF-7



DIVTECH (green) + **miRNA** (red)

Fibroblasts



DIVTECH:peptide (green)

Figure 8. Internalization of blank, miRNA and peptide loaded **DIVTECH** in different cell types.

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THERAPEUTIC DELIVERY OF BIOMOLECULES

DIVTECH can efficiently achieve the intracellular delivery of therapeutic molecules such as proteins and peptides and obtained the desired therapeutic effect. We have evaluated *in vitro* the therapeutic efficiency of a free peptide that can barely enter in the cells, and the peptide associated to two competitors well-positioned in the market and compared to **DIVTECH** (Fig. 10, 11). **DIVTECH** showed a significantly higher therapeutic effect than competitors.

We have also proven that **DIVTECH** can remarkably enhance the internalization of active proteins inside the cells. When compared to competitors, a higher effect (blue signal), and no toxicity of **DIVTECH** is observed. However, the competitors showed high toxicity due to the presence of cationic compounds that can be clearly observed in the pictures as cell debris and a different cell morphology (Fig. 12).

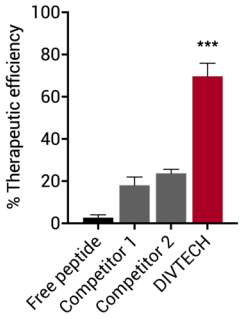
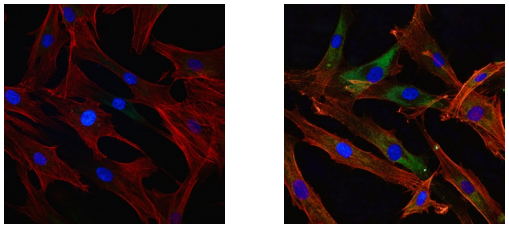
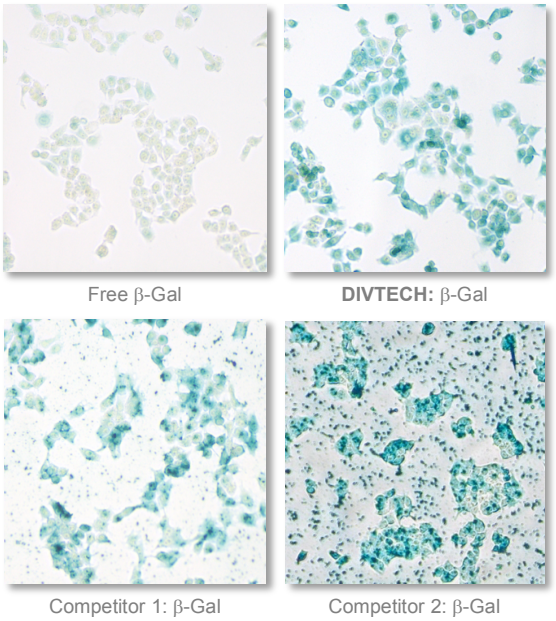


Figure 10. Therapeutic efficiency of **DIVTECH** loaded with a peptide and compared to competitors and the free peptide.



FREE PEPTIDE | **DIVTECH**: PEPTIDE

Figure 11. Intracellular delivery of FITC-labeled peptides to fibroblast as free peptides or using **DIVTECH** delivery system (blue: cell nuclei, red: cytoskeleton, green: PEPTIDE).



Free β-Gal | **DIVTECH:** β-Gal

Competitor 1: β-Gal | Competitor 2: β-Gal

Figure 12. Therapeutic delivery of **DIVTECH** loaded with an active protein (β-Gal enzyme, 540 KDa) and compared to competitors and the free protein in human fibroblasts.

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BIODISTRIBUTION

DIVTECH can be radiolabeled with different radioisotopes for efficiently evaluating the biodistribution of the nanosystems, such as 89Zr (Fig. 14), 68Ga (Fig. 15) or 67Ga (Fig. 16). DIVTECH, without functionalization, can easily accumulate in the liver, spleen, and lungs.

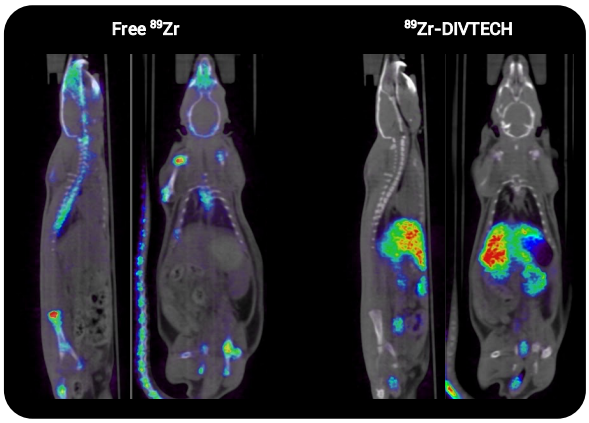

Figure 14. Free ^{89}Zr and ^{89}Zr-labeled **DIVTECH** were intravenously injected in healthy rats.
The biodistribution of the ^{89}Zr was evaluated 22 hours post-injection by PET/CT imaging.

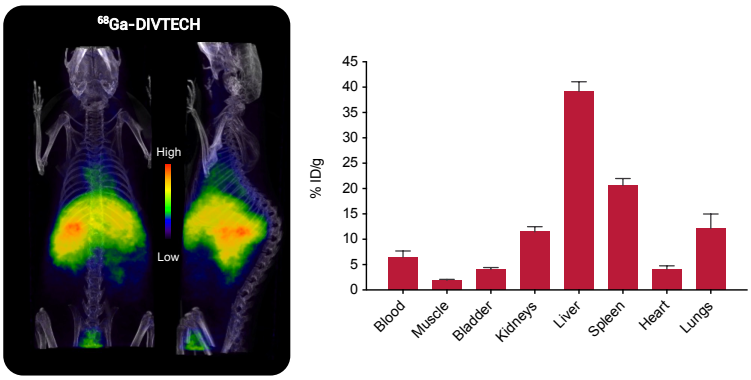

Figure 15. Representative PET/CT whole-body coronal images of ^{68}Ga-**DIVTECH** biodistribution in healthy C57BL/6 mice (n=5). The biodistribution of the ^{68}Ga was evaluated 2 hours post-intravenous injection (a). Ex vivo biostribution of radiolabeled **DIVTECH** 4 hours post-injection (b).

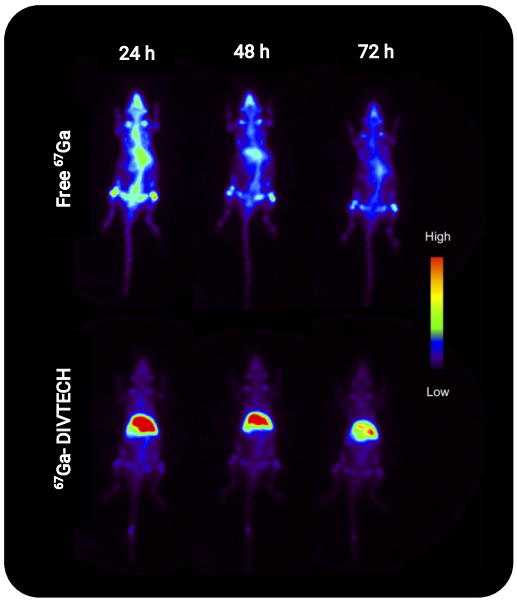

Figure 16. Whole-body SPECT images of ^{67}Ga-**DIVTECH** biodistribution in healthy Sprague-Dawley rats (n=5) compared to free ^{67}Ga during 72 hours after intravenous injection.

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DIVERSA PRODUCT CATALOG

Cat No.	Product	Specification
DIV000F1	DIVTECH FluoGreen Kit	Tracking fluorescent DIVTECH for a positive **cell uptake control**
DIV010	DIVTECH Small Molecule Kit	Enhancing intracellular delivery of **small molecules**
DIV010F1	DIVTECH FluoGreen Small Molecule Kit	Tracking intracellular delivery of **small molecules**
DIV021	DIVTECH Broad Range Peptide Kit	Enhancing intracellular delivery of a broad range of **peptides**
DIV021F1	DIVTECH FluoGreen Broad Range Peptide Kit	Tracking intracellular delivery of a broad range of **peptides**
DIV031	DIVTECH Broad Range Protein Kit	Enhancing intracellular delivery of a broad range of **proteins**
DIV031F1	DIVTECH FluoGreen Broad Range Protein Kit	Tracking intracellular delivery of a broad range of **proteins**
DIV042	DIVTECH Anionic Protein/Peptide Kit	Enhancing intracellular delivery of anionic **proteins/peptides**
DIV042F1	DIVTECH FluoGreen Anionic Protein/Peptide Kit	Tracking intracellular delivery of anionic **proteins/peptides**

To learn more and to order, get in touch with your local Tebubio office.

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