Product Handling Instructions

1. How can I obtain quality control documents for InvivoChem products?

You can download these files from the product webpage of our website (www.invivochem.com) under "Quality Control Documentation" section. InvivoChem 100% guarantees product quality. In order to ensure the high quality and purity of our products, InvivoChem provides a complete set of quality control documents (COA, MSDS, NMR, HPLC, MS, etc.) for each batch of each product.

2. What are the shipping conditions of InvivoChem products?

a) InvivoChem stable products are usually shipped at room temperature, because our products are usually obtained by chemical synthesis, and the synthetic reactions are performed under 50-80 ℃.

b) Liquid or special products may be shipped with blue ice or Cooling Rack in a foam insulated shipping box. InvivoChem products are relatively stable at room temperature for a few weeks during ordinary shipping and time spent in Customs. Their quality will not be affected if the blue ice melts upon receiving, and products can be used with confidence.

c) Temperature sensitive products will be shipped with dry ice.

3. How do I store the product?

Recommended storage conditions and precautions regarding proper product handling can be found in the product web page under the "Physicochemical and Storage Information" section and also in the Certificate of Analysis (COA) document. Here are the general storage guidelines for InvivoChem products: If the solution is stored at -20 ℃ for more than one month, it should be re-examined to ensure its efficacy. Avoid repeated freeze and thaw cycles. Storage conditions for special products should refer to their COAs.

Powder Form	-20°C	3 years
	4°C	2 years
In solvent	-80°C	6 months
	-20°C	1 month

4. How should I do deal with the product after receiving it? (Right before formulation or use)

During transportation, the compound/product may adhere to the neck or cap of the vial. Before opening the vial, please gently tap the vial to let the product fall to the bottom of the vial, then centrifuge to gather the compound at the bottom of the vial. For liquid products, centrifuge the vial at 200-500 RPM to gather the liquid at the bottom of the vial. Try to avoid loss or contamination of products during handling.

5. How can I prepare the compound stock solution?

Select an appropriate solvent for the preparation of stock solution based on your experiment needs. Solubility information is available at the product webpage. For most products, we only offer solubility data in DMSO and/or water. For some products, we may also provide solubility data in a specialized formulation. For solubility in other solvents, please email tech@invivochem.com. If you cannot find the solubility information you are looking for, or you have difficulty dissolving a product in solution, you can also get assistance via the above email. Once the stock solution is prepared, aliquot it to routine usage volumes and store at -20 ℃ or -80 ℃. Avoid repeated freeze and thaw cycles. Molarity Calculator from InvivoChem website is recommended for the related calculation (under the "Tool" tab in the Navigation bar, or at the bottom of the "Physicochemical and Storage Information" section of the product webpage.

6. How do I dilute the compound solution for *in vitro* cellular experiments?

Stock solution prepared in ddH2O (Double distilled water) can be directly diluted with medium to prepare the working solution.

When DMSO is used to prepare the stock solution, the stock solution is diluted in the culture medium to prepare a working solution. Make sure the concentration of DMSO is <0.5% to avoid poisoning the cells. A negative control in the experiment is usually the culture medium with DMSO at the same concentration. It is recommended that the dilution process should be performed in a stepwise manner to avoid compound precipitation due to fast change of concentrations.

Products are for research use only • Not for human or veterinary use

InvivoChem LLC

Tel: +1 708 310-1919 **Fax: +1 708 557-7486** **Email: info@invivochem.com**

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Our products are mainly lipophilic/liposoluble, thus it is common that there is precipitation when diluted by the cell culture medium. However, they can be completely dissolved by ultrasonication.

7. How do I use the products for *in vivo* animal experiments? e.g. route of administration, dosage, drug formulation, and administration frequency?

Stock solution prepared in ddH2O can be directly diluted with PBS or 0.9% NaCl to prepare the working solution.

Stock solution prepared in DMSO can also be diluted with PBS or 0.9% NaCl to prepare the working solution. In order to reduce the toxicity of solvents (e.g. DMSO) to animals, the final concentration of DMSO in the working solution should preferably be $\leq$ 2%. When a product precipitates out during the dilution process due to their low water solubility, you may use co-solvents to facilitate the dissolution of the compounds. Common co-solvents include glycerol, Tween 80, cyclodextrin, carboxymethyl cellulose sodium (CMC-Na), PEG400, etc. A suspension may also be used for oral or intraperitoneal injection. It may not influence the efficacy of the products by gavage using suspensions or emulsions. For hydrophobic drugs, lipid formulations may also be used. Please send an email to tech@invivochem.com if you need further assistance. Routes of administration, dosage, drug formulation, and administration frequency reported in publications may be available at our product webpage. **Please note** that InvivoChem has not independently validated the accuracy of those methods and they are listed for reference only. InvivoChem strongly recommends customers to read literature methods/protocols carefully before determine which protocol you should use for *in vivo* studies.

Recommended Administration volumes (and possible maximal dose volumes):

Species	Route of Admin. and dose volumes (mL/kg)					
	Oral	SC	IP	IM	IV-bolus	IV-slow inj
Mouse	10 (50)	10 (40)	20 (80)	0.05 (0.1)	5	(25)
Rat	10 (40)	5 (10)	10 (20)	0.1 (0.2)	5	(20)
Rabbit	10 (15)	1 (2)	5 (20)	0.25 (0.5)	2	(10)
Dog	5 (15)	1 (2)	1 (20)	0.25 (0.5)	2.5	(5)

Dose conversion between animals based on BSA (based on FDA Guidelines):

Species	Mouse	Rat	Rabbit	Dog	Hamster	Guinea pig
Weight (kg)	0.02	0.15	1.8	10	0.08	0.4
Body Surface Area (BSA; m^2)	0.007	0.025	0.15	0.5	0.02	0.05
K_m factor	3	6	12	20	5	8

Animal A (mg/kg) = Animal B (mg/kg) multiplied by (Km of Animal B) / (Km of Animal A)

For instance, to modify the dose of drug X used for a mouse (10 mg/kg) to a dose based on the BSA for a rat, multiply 10 mg/kg by the Km factor (3) for a mouse and then divide by the Km factor (6) for a rat. This calculation provides the rat equivalent dose for drug X as 5 mg/kg.

Rat dose (mg/kg) = mouse dose (10 mg/kg) × (Km of mouse) / (Km of rat) = 5 mg/kg

8. Are InvivoChem products/compounds sterile?

Because DMSO is typically used to prepare stock solutions for InvivoChem products, and DMSO itself is strongly bactericidal and will not introduce bacteria to compounds. However, it is important to keep the operating environments and the instruments sterilized before experiments. Compounds can also be sterilized by filtration prior to use depending on specific experimental requirements. High temperature and high pressure sterilization are **NOT** recommended.

9. What is the range of the weighing errors for InvivoChem products?

We use analytical balances (Precision = 0.01 mg) to weigh the products with high accuracy. For small size products (≤25 mg), e.g. 1mg, 5mg, 10mg, you can directly dissolve it in a pre-calculated amount of solvents (e.g. DMSO), to prepare the stock solution to a desired concentration (e.g. 10 mM or 20 mM). Please DONOT re-weigh the compound to avoid the loss of products during the re-weighing process.

Weighing range	Error range
1-5 mg	0.05-0.1 mg
5-25 mg	0.1 mg
50-500mg	1 mg
>1g	2-5 mg

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Frequently Asked Questions

Question #1: How stable is a compound during transportation and how should I store a compound?
Answer: Unless specified, most products are stable at ambient temperature for a few weeks during ordinary shipping and time spent in Customs. Please store the compound at 0-4 ℃ for short term and -20 ℃ for long term use.

Question #2: How stable is a compound in cell culture media? Do I need to change media every day?
Answer: Small molecule compounds are generally stable in cell culture media for at least a couple of days. In most cases, a chemical compound is freshly added to the media each time when you reconstitute or change the culture condition. In general, you do not need to change media every day unless it is clearly specified by researchers in peer-reviewed publications, or you observed a decreased effects after one day.

Question #3: Are the compounds provided by InvivoChem sensitive to freeze-thaw cycles?
Answer: Different from proteins, peptides or other biomacromolecules, most of our small molecule compounds are not sensitive to freeze-thaw cycle unless specified. The stock solutions of a typical small molecule compound (e.g., in DMSO) can be frozen and thawed multiple times. However, we recommend you to aliquot your DMSO stock solutions (e.g. 10 mM) into 50 or 100 μL aliquots if you intend to freeze them for long term use. In some cases, a chemical compound may precipitate out at low temperature, and they have to be re-dissolved fully after warming up with tapping or vortexing of the sample tube or vial gently.

Question #4: How can I make stock solutions or formulate a compound for in vivo animal studies?
Answer: listed below are formulations commonly used for i.p injections and oral administrations:

Common formulations for i.p. injections	Common formulations for oral gavage
1. DMSO : Tween 80： Saline=10 : 5 : 85 2. DMSO : PEG300： Tween 80 : Saline =10 : 40 : 5 : 45 3. 2-Hydroxypropyl-β-cyclodextrin : Saline=50 : 50 4. DMSO : PEG : castor oil : Saline=5 : 10 : 20 : 65 5. Ethanol : Cremophor : Saline =10： 10 : 80 6. Dissolve in Cremophor/Ethanol (50 : 50), then diluted by Saline	1. Suspend in 0.2% Carboxymethyl cellulose 2. Dissolved in PEG400 3. Suspend in 0.5% CMC Na 4. Dissolve in 0.25%Tween80 and 0.5% carboxymethyl cellulose 5. Suspend in 0.5% Carboxymethyl cellulose 6. Mixing with food powders

Please note that the above protocols are for reference only. InvivoChem strongly recommends customers to read literature methods/protocols carefully before determine which protocol you should use for in vivo studies, as different compounds have different solubility properties and have to be formulated differently.

Question #5: What is the stock concentration of a compound (e.g. in DMSO) do you recommend to use?
Answer: Usually 10 mM or 20 mM. For preparing DMSO stock solutions, we suggest you to prepare a 10 mM or higher concentrations of a compound, as a more than 1000x dilution would be applied typically when making final concentrations of a compound in cell culture media, this is to minimize any solvent effects on your cells (typically less than 0.5% of DMSO might be used for most of cells).
Typically<0.5-1% DMSO is tolerable by most cancer cells in culture media. For more sensitive cells such as primary cells, try to use <0.1% DMSO in the cell culture media.

Question #6: What should I do if I see precipitation in a DMSO solution of a compound?
Answer: If you see a compound precipitated out in a DMSO solution, you need to re-dissolve it fully upon warming up and tapping or vortexing the sample tube or vial gently.

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Guidelines for Dissolving Peptides

Identifying a suitable solvent or formulation for and/or determining the solubility of a given peptide is a highly challenging task as improper solubilization may lead to loss of peptides or even failure of an experiment. The steps illustrated below provides you with a method to perform a solubility test for determining the best solvent for a synthetic peptide. **It is best to test it by dissolving a minute amount of peptide, rather than the entire sample.** As a general rule, peptides should first be dissolved in distilled/sterile water, in particular peptides of fewer than five amino acid residues. For individual peptides, conditions are chosen for optimum solubility based on the given peptide sequence.
In general, hydrophilic peptides dissolve well in water (H_2O), but hydrophobic peptides may not be soluble in water.

Guidelines for Peptide Solubility and Storage:
1. Calculate the length of the peptide.

2. Calculate the overall charge of the peptide following the instructions in the table below:

Types of Amino acids	Amino acids and abbreviations	Charge Value Assigned
Acidic amino acids	Asp (D), Glu (E), C-terminal -COOH	-1
Basic amino acids	Arg (R), Lys (K), His (H), N-terminal -NH2	+1
Neutral amino acids	Gly (G), Ala (A), Leu (L), Ile (I), Val (V), Cys (C), Met (M), Thr (T), Ser (S), Phe (F), Tyr (Y), Trp (W), Pro (P), Asn (N), Gln (Q)	0

3. How to choose solvents for dissolving peptides based on their overall charges and hydrophilicity/hydrophobicity:

Overall charge of peptides	Protocol for dissolving peptides
Negatively charged peptides (<0)	1. First, try to dissolve the peptides in water; 2. If insoluble in water, add a small amount of NH_4OH (e.g.<50 μL); 3. If still insoluble, add a small amount of DMSO (e.g. 50-100 μL) to aid the solubilization of the peptide.
Positively charged peptides (>0)	1. First, try to dissolve the peptides in water; 2. If insoluble in water, try to dissolve the peptide in 10%-30% acetic acid solution (10%-30% acetic acid in water, w/w); 3. If still insoluble, try to dissolve the peptide in a small amount of DMSO (e.g. 50-100 μL).
Zero charged peptides(=0)	1. First, try to dissolve the peptide in organic solvents (e.g. acetonitrile, methanol); 2. For highly hydrophobic peptides, try to dissolve in a small amount of DMSO, followed by dilution of the solution with water to the desired concentrations.

4. Storage information:

Powder form	-80 ℃ for 2 years (seal the vial well, protect from moisture)
	-20 ℃ for 1 year (seal the vial well, protect from moisture)
Solution form	-80 ℃ for 6 months (seal the vial well, protect from moisture)
	-20 ℃ for 1 month (seal the vial well, protect from moisture)

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