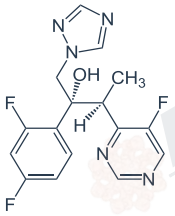
APExBIO
Achieve Perfection, Explore the Unknown



General User Guide for APExBIO Small Molecule Compounds

The general user guide is for reference only; please optimize the conditions according to actual experimental requirements

1 What items are included in the package you received?

Each shipment typically includes a foam insulation box, a delivery note, the Certificates of Analysis (COA), ice packs or dry ice and ordered products. Upon receipt of your shipment, please verify that all items listed on the delivery note are present. If any items are missing or damaged, please contact us immediately (support@apexbt.com). We will address your issue as quickly as possible.

Product Datasheets and COA are available on the respective product pages on our official website (https://www.apexbt.com).

2 What if the ice packs/dry ice delivered with the products have melted/evaporated upon receipt?

It is not uncommon for ice packs to be partially or fully melted, or for dry ice to have evaporated during transit.

- Most of our products are stable at ambient (room) temperature for short periods.
- Ice packs and dry ice are included primarily to prevent exposure to extreme or fluctuating temperatures during shipping.
- Low temperatures are generally required only for long-term storage, not for short-term transportation.
- For temperature-sensitive products, specialized packaging is used to maintain product integrity.

Therefore, any changes in the conditions of ice packs/dry ice upon arrival does not necessarily indicate compromised product quality.

3 What are the recommended storage and handling conditions for the received products?

1) Powder Form (Recommended for Long-Term Storage)

For optimal stability, we recommend storing products in powder (lyophilized or solid) form whenever possible.

Small molecule compounds (powder):
Stable for **2–3 years at -20 °C**.
Stable for **1–2 years at 4 °C**.
Peptide products (powder):
Stable for **1–2 years at -20 °C**.
Stable for **~1 year at 4 °C**.

2) Solution Preparation and Storage

- Prepare working solutions **freshly prior to use** whenever feasible.
- **For stock solutions**:
  - **Aliquot** into appropriate volumes to avoid repeated freeze–thaw
  - cycles
    Seal tightly to avoid contamination and evaporation

*Impact of freeze–thaw cycles:

Occasional freeze–thaw cycles generally **do not significantly affect most small molecule compounds**. However, repeated freeze-thaw cycles may reduce activity, especially for sensitive compounds.

3) Stability of Stock Solutions

Small molecule compounds (stock solution):
Stable for **1 month at -20 °C**.
Peptide products (stock solution):
Stable for up to **1 week at -20 °C**.
Longer storage is not recommended due to potential degradation.

4) Special Substance Handling

a) **Waxy, oily and viscous compounds** are difficult to weigh accurately. It is recommended to dissolve the compound directly in an appropriate solvent.
b) **Hygroscopic compounds** should be stored in desiccators.

Note:
- Strictly follow storage conditions specified in the COA or datasheet.
- Activity testing is recommended after extended storage.
- For highly unstable compounds in solution form (e.g., fluorescent probes, peptides), the stock solution should be prepared freshly before use.

4 What is the weighing error range for the products?

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

5 Is pre-treatment required prior to use?

1) Centrifugation Before Opening:

- Briefly centrifuge at low speed before opening to collect material at the bottom.
- For glass vials containing small volumes, do not centrifuge, as it may cause vial cracking. In such cases:
  - Add solvent directly into the vial.
  - Gently mix by **vortexing or ultrasonication in water bath.**

2) Aliquoting and Handling:

- Aliquot products according to routine experimental usage **(see Section 3)**.
- Products dissolved in volatile organic solvents (e.g., ethanol) should be used promptly to avoid the effect of solvent evaporation.
- For products that are unstable in solution form, handling and storage of solutions should follow **Section 3**.

6 Is sterilization required for the products?

Most small molecule compounds are dissolved in **DMSO or aqueous**

solutions, and sterilization requirements depend on the solvent system.

1) DMSO-Based Solutions:

- DMSO solutions are generally considered **self-sterilizing** due to the anti-bacterial properties of DMSO.
- Ensure that the experimental environment and equipment are sterile.
- Prepare a 1000× stock solution in DMSO prior to use.
- If contamination is suspected in the working solution, perform sterile filtration before use.

2) Aqueous Solutions:

- When using water as a solvent, prepare a ≥50X stock solution using **sterile water**.
- Dilute to working concentration prior to use. The working solution should be **sterilized by filtration**, using a 0.22 μm sterile filter membrane.
- Prior to filtration, ensure the solution is clear and free of visible precipitation (microscopic inspection is recommended).
- Do not autoclave solutions, as this may compromise compound integrity.

7 How to dissolve lyophilized peptide powder?

Appropriate solvent selection is critical and should be based on peptide physicochemical properties:

1) **Acidic or neutral peptides:** Dissolve in 0.1 M ammonium bicarbonate, then dilute with water
2) **Basic peptides:** Dissolve in 25% acetic acid, then dilute with water
3) **Hydrophobic peptides:** Dissolve in organic solvents such as acetonitrile (ACN), DMSO, or DMF
4) **Peptides containing oxidation-sensitive residues (e.g., Trp, Met, Cys):** Dissolve in oxygen-free (anaerobic) water or in the presence of reducing agents to prevent oxidation.

Note:
Due to limited stability of peptide solutions **(see Section 3)**, It is strongly recommended to **prepare solutions immediately before use**.

8 How to prepare stock solution?

1) Solvent Selection:

Most small molecule compounds are soluble in organic solvents such as DMSO and ethanol, but poorly soluble in aqueous solution (e.g., water, PBS, saline).

Please select solvents according to solubility information provided on the product pages of our official website (https://www.apexbt.com)

Interpretation of solubility data (example):
≥1.6 mg/mL in DMSO; Insoluble in H_2O; ≥85.9 mg/mL in EtOH

- **≥1.6 mg/mL in DMSO:**
At least 1.6 mg can be dissolved in 1 mL of DMSO under tested conditions. Saturated solubility is unknown.

- **Insoluble in H_2O:**
The compound is insoluble or only minimally soluble in water.

- **≥85.9 mg/mL in EtOH:**
At least 85.9 mg can be dissolved in 1 mL of ethanol under tested conditions. Saturated solubility is unknown.

2) How to facilitate Dissolution?

- For compounds with limited solubility, incubate at 37 °C or use an ultrasonic water bath to promote dissolution. If precipitation persists, repeat incubation at 37 °C or ultrasonication as needed.
- Cooling or freezing may induce reversible precipitation. If precipitation is observed after storage, ensure the solution is fully re-dissolved (e.g., by vortexing, gentle warming, or ultrasonication) prior to use.
- If poor solubility is observed, add appropriate **co-solvents (e.g., Tween 80, glycerol, sodium carboxymethyl cellulose (CMC-Na), PEG400 etc.)** If any difficulties persist, please contact technical support prior to proceeding with experiments (support@apexbt.com).

3) Key Notes:

a) The APExBIO molarity calculator is available to assist in concentration calculations (https://www.apexbt.com/molarity-calculator).

b) Ensure **thorough mixing** during solution preparation.

c) Aliquot after preparation before storage **(see Section 3)**.

d) For compounds unstable in solution, handling and storage of solutions should strictly follow **Section 3**.

e) **Moisture-contaminated DMSO** may reduce solubility. Use fresh, anhydrous DMSO.

f) For compounds with extremely low solubility, using suspensions is acceptable. For oral or intraperitoneal administration, suspensions generally do not significantly affect compound activity.

g) If you have any other questions, please contact APExBIO technical support before performing the experiment (support@apexbt.com).

9 How to prepare working solution?

1) Dilution from Organic Solvent Stock (e.g., DMSO):

- Ensure that the compound is fully dissolved in the final system.
- Ensure that the organic solvent concentration in the working solution remains within biologically acceptable limits **(see Section 10)**.
- If precipitation occurs during dilution, re-dissolve following **Section 8**.
- Using stepwise dilution approaches (e.g., gradual 10-fold dilution) can also avoid precipitation. The key principle of stepwise dilution is to gradually reduce the proportion of organic solvent and avoid abrupt environmental changes that may cause precipitation.

2) Dilution from Aqueous Stock Solutions:

For compounds soluble in water, stock solutions can be directly diluted to working concentrations using cell culture medium, PBS or ddH2O. Ensure thorough mixing during solution preparation.

10 How to use the products in cell and animal experiments?

1) Cell-Based Experiments:

For stock solutions prepared in aqueous solvents (e.g., ultrapure water), dilute directly to the desired working concentration with culture medium or PBS.

For stock solutions prepared in DMSO, while diluting to working concentration with culture medium or PBS, ensure that the final concentration of DMSO **does not exceed 0.5% (v/v)** to minimize cytotoxicity.

2) Animal Experiments:

For stock solutions prepared in DMSO, dilute to working concentration using saline or PBS. Ensure that the final DMSO concentration **does not exceed 2% (v/v) to avoid toxicity.**

If precipitation occurs during dilution, re-dissolve following **Section 8**. For compounds with extremely low solubility, suspensions are acceptable.

Administration volumes considered optimal practice (and possible maximal dose volumes mL):

Species	Route and volumes (mL/kg)					
	Oral	s.c.	i.p.	i.m.	i.v. (bolus)	i.v. (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)

Notes:

a) **Values provided in brackets** represent the maximum allowable administration volume for a given route. Excessive dosing volume may induce adverse effects (e.g., vomiting, abdominal distension). Optimal (lower) volumes are recommended to minimize animal stress.

Example: For mice, 10 mL/kg (oral) is preferred over higher volumes (e.g., 50 mL/kg).

b) **When increasing dose levels due to experimental requirements** (e.g., poor solubility), evaluate dose tolerability carefully, increase dose gradually toward the upper limit and include appropriate control groups to monitor adverse effects.

c) **Physiological characteristics vary across species** (e.g., mouse abdominal capacity is limited, whereas rats have more developed musculature) and affect dose volume and tolerability. Experimental design should consider species-specific parameters.

d) For further guidance, see ***Dihl KH, et al. J Appl Toxicol, 2001, 21(1), 15–23.***

Conversion of different model animals based on Body Surface Area (for reference only)

Species	Body Weight (kg)	Body Surface Area (m^2)	Km Coefficient
Baboon	12	0.6	20
Dog	10	0.5	20
Monkey	3	0.24	12
Rabbit	1.8	0.15	8
Guinea Pig	0.4	0.05	6
Rat	0.15	0.025	6
Hamster	0.08	0.02	5
Mouse	0.02	0.007	3

***Animal A (mg/kg)=Animal B (mg/kg)×(Km of Animal B/Km of Animal A)**
Value based on data from FDA Draft Guidelines: ***Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers*** (U.S. Food and Drug Administration, Rockville, Maryland, USA, 2002).

***Notes: Why Km coefficient is important?**

- The **Km coefficient** represents the ratio of **body surface area (BSA) to body weight.**
- Pharmacokinetics and toxicity are often more closely correlated with **dose per unit BSA** rather than body weight.
- BSA correlates with the area of drug interacting with the external environment (e.g., dermal absorption, respiratory metabolism).
- Direct conversion based solely on body weight may lead to inaccurate dosing:

  Example: A rat weighs ~6× more than a mouse, but its BSA is only ~2–3× greater. Simple weight-based scaling may therefore result in overdosing.

3) Key Notes:

a) Experimental conditions **should not be determined based on a single publication**. Perform pilot studies to optimize parameters such as:
- Animal strain, age
- Dose, frequency, treatment duration
- Sampling time points and endpoints.

b) Always include a **vehicle control** group (e.g., DMSO or corresponding solvent).

c) For technical questions, please contact APExBIO technical support prior to experimentation (support@apexbt.com).

11 Product categories

Compound Libraries | **Inhibitors** | **Amino Acids**
Assay Kits | **Dyes** | **Proteins**
Peptides | **Cytokines** | **Natural Products**

12 Contact Us

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