TargetMol®
YOUR TARGET MOLECULES

User Guide of Recombinant Proteins

1 How are recombinant proteins shipped?

Most recombinant proteins are delivered as lyophilized powder, which are insensitive to temperature change and are stable at room temperature. They are typically shipped at room temperature or with blue ice. For liquid recombinant proteins, dry ice is generally used for shipping. It is normal if the product is arrived with a melted ice pack. The protein can still be used without any negative impact on its quality for a short period. If a specific protein is unstable at room temperature, appropriate cold chain shipping will be arranged to ensure product quality remains intact.

2 How should recombinant proteins be stored?

It is recommended to store recombinant proteins at -20°C to -80°C for future use.
Lyophilized powders can be stably stored for over 12 months, while liquid products can be stored for 6-12 months at -80°C. For reconstituted protein solutions, they should be used promptly. If they cannot be used up within one week, the product can be stored at -20°C to -80°C for at least 3 months. Avoid multiple freeze-thaw cycles and store products in aliquots. When aliquoting and freezing, it is advisable to use a solution or medium with a certain concentration of carrier protein for reconstitution. The product concentration should not be less than 100 μg/mL, and the volume per vial should be no less than 20 μL.

3 Why are carrier proteins needed in some recombinant protein solutions?

Carrier proteins help improve the stability of proteins and prevent them from adhering to the walls upon freeze and thaw. If proteins adhere to the tube, it will lead to a decrease in protein concentration in the solution which affects protein activity. For long term storage, carrier proteins, such as 0.1% Bovine Serum Albumin (BSA), 5% Human Serum Albumin (HAS), 10% Fetal Bovine Serum (FBS), or 5% trehalose, are used to reduce such loss. If the concentration of the recombinant protein is low, please add an appropriate amount of carrier protein to maintain protein activity.

4 How to reconstitute recombinant proteins?

Solvents required for reconstitution may vary. In general, most proteins can be reconstituted by simply adding sterile distilled water, while a few may require alternative solvents. To ensure a proper reconstitution process, select the appropriate solvent according to the product manual.

1) After opening the tube, quickly add specific reconstitution buffer to the lyophilized powder. Invert the sample gently a few times and avoid vigorous shaking.

Note：If you receive a centrifuge tube, centrifuge at 10,000-12,000 rpm for 20-30 seconds before opening the tube to collect the lyophilized powder adhering to the cap or tube well.

2) Leave the reconstituted sample at room temperature for several minutes to ensure complete dissolution of the protein.

3) For products containing glycerol that are difficult to dissolve, they usually adhere as a viscous form to the tube wall after lyophilization. After adding the reconstitution buffer, aspirate the reconstitution solution with the pipette and repeatedly wash the sample from the tube wall. Invert the sample gently until a clear solution is obtained. Avoid vigorous shaking.

4) Proceed with your experiments or aliquot for storage. For short-term experiments, store the solution at 2 °Cto 8 °C and use it up within a week. For long-term storage, it is recommended to add carrier proteins or a certain concentration of culture medium in the aliquots before storing at -20 °Cto -80 °C. If experiments require no serum, a 5% trehalose solution can be used as a carrier.

5 Why are some products not obviously visible?

During transportation, a small amount of lyophilized powder may adhere to the vial walls or cap due to vibrations or turbulence, causing the powder hard to be observed. In addition, the lyophilization may take on various shapes that are easily skipped by the eye as the volume being influenced by various factors, including the composition of the buffer prior to lyophilization, salt ion concentration, and the concentration of the product itself. Thus, please remember to centrifuge and reconstitute the product to the recommended concentration upon arrival to ensure the quality and performance.

6 How are recombinant proteins tested in QC?

Methods to determine the purity:

1) SDS-PAGE assay 2) HPLC assay 3) Silver staining assay

Methods to determine the concentration:

1) Bradford protein quantification assay 2) BCA protein quantification assay 3) SDS-PAGE protein quantification assay

7 How to measure recombinant protein activity?

ED_{50} is defined as the protein concentration at which the activity is 50% of the maximum response and is reported in ng/mL. The formula is as follows:

$$\frac{\text{units}}{\text{mg}} = \frac{1 \times 10^6}{ED_{50}\,(\text{ng/mL})}$$

Please note that there is not a way to convert between these “International Units (IU)” and the ED_{50}.

8 Is there batch-to-batch variation in the ED_{50} value of recombinant proteins?

The ED_{50} value of recombinant proteins may vary between different batches or when using different experimental methods. The ED_{50} value measured in the Certificate of Analysis (COA) may not necessarily be applicable to every experimental condition. In any case that the protein is being used in experimental conditions that differs from the COA, it is recommended to obtain an ED_{50} value according the actual experimental conditions.

9 What are the common species for recombinant proteins?

Human	Rat	Rabbit	Porcine	Cat	Cynomolgus	Virus
Mouse	Bovine	Sheep	Canine	Rhesus Macaque	Xenopus laevis	Others

10 Do recombinant proteins show cross-species activity?

Recombinant proteins from different species may display different degrees of cross-species activity. Many human-derived cytokines are effective in mouse cell lines, and mouse-derived cytokins can also affect human cells but their specificity may not be strong. Highly conserved factors like Fibroblast Growth Factors (FGFs), Neurotrophic Factors (e.g., BDNGF, GDNF, CNTF, β-NGF), and Transforming Growth Factor-β (TGF-β) exhibit strong cross-species activity.

However, some cytokines are species-specific, such as Interferons, Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Interleukin-3 (IL-3), and Interleukin-4 (IL-4). For these species-specific factors, they may only produce the expected biological effects on cells of the corresponding species.

Therefore, it is recommended to select recombinant proteins from the species corresponding to specific assays or application needs.

11 What are the different expression systems for recombinant proteins?

What are the different expression systems for recombinant proteins?

Recombinant protein expression systems primarily include prokaryotic expression systems (mainly including *Escherichia coli/Bacillus subtilis* expression system) and eukaryotic expression systems (mainly including Mammalian cell/Yeast/Insect expression system).

1) **Prokaryotic Expression Systems:** Prokaryotic expression systems include *E. coli* expression system, *Bacillus* expression system, and *Streptomyces* expression system, among which the most commonly used is *E. coli* expression system. Prokaryotic expression system is advantageous of being cost-effective, rapid and high-yielding, and is widely used. However, it lacks post-translational modifications, is not suitable for expressing large molecule proteins and is easy to form inclusion bodies.

2) **Yeast expression systems:** Yeasts commonly used for the expression of exogenous proteins include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Pichia pastoris*, and *Kluyveromyces lactis*. Yeast expression systems are cost-effective, rapid, and offer high yields. They allow for some level of post-translational modifications. However, they are glycosylated in a mannan-type manner, unlike mammalian cells.

3) **Insect cell expression systems:** Proteins that are challenging to express in bacterial systems, such as membrane proteins, large proteins, and protein kinases, can be successfully expressed in insect cells, such as Sf9 or High-5 cells. The insect cell expression system is advantageous with large gene capacity, suitable for expressing toxic proteins, with mammalian-like characteristics, and short vector construction cycles. It allows for post-translational modifications but lacks some glycosylations. It's also worth noting that recombinant proteins may involve degradation issues in the baculovirus expression vector system (BEVS).

4) **Mammalian cell expression systems:** Mammalian cells are typically used to express exogenous proteins when target proteins contain complex disulfide bonds or when post-translational modifications cannot be expressed using other expression systems. Some commonly used mammalian cell lines for protein expression include Hela cells, Human embryonic kidney 293 cells (HEK293) and Chinese hamster ovary cells (CHO). Mammalian cell expression systems are advantageous with lower levels of endotoxins, better activity, and superior post-translational modifications that allow for transient and stable transfections, but the time required for highly expressed cell lines is long and the cost of scale-up culture is relatively high.

12 How to choose the appropriate expression system?

Purpose	Expression System Recommendation
For generating specific antibodies using proteins as antigens and native activity or protein modifications are not needed.	Prokaryotic expression systems
For protein crystallization and structural studies which require high-purity recombinant proteins.	Prokaryotic expression systems Insect expression systems Mammalian cell expression systems
For functional studies which require to maintain the protein's native activity.	Prokaryotic expression systems Eukaryotic expression systems
For proteins intended for drug research that must maintain sequence integrity and native activity.	Eukaryotic expression systems

14 How do different tags affect recombinant proteins?

Protein tags refer to proteins or peptides that are expressed in fusion with the target protein to facilitate the expression, detection, tracing and purification of the target protein.

1) Small molecular weight peptides, such as His (0.84 kDa), Flag (1.01 kDa), Myc (1.20 kDa), have small molecular weight and usually have little effects on the structure and function of the target protein, which are convenient for purification, expression and related research.

2) Solubility-enhancing fusion proteins, such as GST (26 kDa), Sumo (12 kDa), MBP (40 kDa) can enhance the solubility of the target protein, protect the recombinant protein from degradation by extracellular proteases and thus improve protein stability, but may affect the protein function.

3) Reporter fusion proteins, such as eGFP (excitation wavelength 488 nm, emission wavelength 507 nm), eYFP (excitation wavelength 513 nm, emission wavelength 527 nm), eCFP (excitation wavelength 433 nm or 453 nm, emission wavelength 475 nm or 501 nm), emits spontaneous fluorescence with high efficiency, high sensitivity and good reproducibility. They are widely used in various applications, including functional localization of proteins, migration changes, expression regulation, transgenic function research, and high-throughput drug screening.