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User guide for Recombinant Proteins

1 How many kinds of recombinant proteins does MCE offer?

A. Classified by protein types: Cytokines and Growth Factors, Immune Checkpoint Proteins, CAR-T related Proteins, CD Antigens, Fc Receptor Proteins, Receptor Proteins, Enzymes & Regulators, Complement System, Ubiquitin Related Proteins, Viral Proteins, Biotinylated Proteins, Fluorescent-labeled Recombinant Proteins, GMP-grade Proteins and Animal-free Recombinant Proteins and others.

B. Classified by species: Human, Mouse, Rat, Rhesus Macaque, Procine, Canine, Bovine, Sheep, Rabbit, Cat, Virus, Xenopus laevis and Others.

2 How many protein expression systems does MCE offer?

MCE provides proteins expressed in *E. coli*, yeast, insect, mammalian, and *E. coli* Cell-free expression systems.

3 How to determine protein purity and quantity?

Methods for purity determination: a. SDS-PAGE; b. HPLC; c. Silver staining.

Methods for protein concentration determination: a. Branford protein assay; b. BCA protein assay; c. Intensity measurement on the SDS-PAGE gel with a BSA standard curve.

4 What are the shipping condition for recombinant proteins?

Most recombinant proteins are shipped with blue ice or in room temperature. Lyophilization increases product stability and reduces packing materials and shipping expenses. The data of quality control tests indicates that lyophilized products shipped at ambient temperature would retain full activity when delivered promptly and stored properly.

5 How are MCE recombinant proteins produced? What are the advantages of eukaryotic expression (yeast, insect and mammalian cells) exist versus expression from *E. coli*?

Most of our recombinant proteins are produced in eukaryotic systems, but the certificate of analysis will provide you with the specific information on how individual products were produced.

Eukaryotes have several advantages as an expression system.

a. Eukaryotes inherently secrete very few native proteins, but they do secrete recombinant proteins. Therefore secretion is typically used as the first step in the purification process from a eukaryotic system. Recombinant proteins made in E. coli are often localized to inclusion bodies. Purification from inclusion bodies can require harsh conditions to free the recombinant protein, followed by subsequent refolding processes. These harsh treatments can negatively affect the function of the protein.

b. Recombinant proteins secreted by eukaryotes are processed by the Golgi apparatus, and thus they can be post-translationally modified. These modifications include glycosylation, phosphorylation and sulfation. There are many proteins that require modifications for protein function, proper folding or solubility.

c. Eukaryotes do not have a bacterial cell wall like *E. coli*, thus there is no endotoxin (lipopolysaccharide) present that can affect inflammatory responses in your target system.

6 When I opened the vial, I didn't see anything. How do I know there is protein in the vial?

Centrifuge the vial prior to opening! Most of our products are lyophilized with a low concentration buffer, so the few micrograms of product may not be very visible. It is recommended that centrifuge the vial in a micro-centrifuge for 20-30 seconds before opening. The accuracy of protein amount is guaranteed by our quality control procedures.

7 I want to try to do an experiment with your protein, but the bioassay you use for determining activity is not the same as my application. Will my application work with your protein?

1. Centrifuge the tube before opening

During shipment, the protein may adhere to the wall or cap of the vial. Before opening the vial, please centrifuge at 10000-12000 rpm for 30 seconds to gather the protein at the bottom of the vial. If a high-speed centrifuge is not available, please centrifuge at 3000-3500 rpm for 5 mins.

2. After centrifugation, add the reconstitution buffer to the lyophilized protein powder and mix gently by pipetting. Resuspend in the reconstitution buffer to recommended concentration (no less than 100 μg/mL).

Note: Vigorous vortexing should be avoided as it can cause protein foaming and denaturation, thereby affecting the protein activity.

3. Once reconstituted, recombinant proteins can be stored no more than a week at 2-8°C.

For experiments with a short cycle (no more than 7 days), the recombinant protein solution can be directly added to the culture system and used up within a week. It is not recommended to freeze the reconstituted product directly at -20°C to -80°C. If the experimental concentration is lower than the reconstituted concentration, dilution can be done with a solution containing carrier proteins.

4. For long-term storage, the protein solution should be diluted further with carrier proteins (0.1% BSA, 5% HAS, 10% FBS or 5% trehalose), and then aliquot and stored at -20°C to -80°C.

Note: Avoid repeated freeze/thaw cycles. Each freeze/thaw cycle will cause denaturation or conformational changes in some proteins, thereby reducing the binding ability of antibodies and accelerating protein degradation.

8 How should I store recombinant proteins? What is the shelf life of your recombinant proteins?

Storage & Stability	Product Form	Temperature	Storage Time
	Liquid	-20 ºC～-80 ºC	1 year
	Lyophilized	-20 ºC～-80ºC	2 years
	Reconstituted	2 ºC～8 ºC	1 week
	Reconstituted (with carrier protein)	-20ºC～-80ºC	3 to 6 months
	Note: 1. For long-term storage, the protein solution should be diluted further with carrier proteins. 2. Please keep in mind that every freeze/thaw cycle may cause some denaturation of the protein.		

9 What is the carrier protein?

Arrier proteins such as HSA or BSA are used to improve the stability of the reconstituted proteins, and help to avoid the product sticking to the walls of the vial. Some recombinant proteins may stick to the plastic tube wall easily, which results in a lower concentration of protein in the solution and ultimately reduces its activity. Carrier proteins can prevent products from sticking to the tube wall by pre-blocking the protein binding site. Therefore, for long-term storage, cytokines or proteins should be further diluted with the solution containing carrier proteins before making aliquots and freezing.

10 Why are some proteins fused to tags? Do protein tags affect protein activity?

Most MCE recombinant proteins are tag-free. Protein tags, however, are useful for several different purposes.

a. Protein tags are useful for protein purification.

b. Tags are used for protein detection in Western blot or ELISA when the specific antibodies are not available.

c. The Fc tag stabilizes molecules, which may increase the half-life of the linked products. Since the Fc fragment tends to dimerize, it helps the linked protein, particularly receptors, to form biologically active dimers.

Protein tags may or may not affect the protein's activity. For some applications, small tags, such as the His-tag, may not affect protein activity and do not need to be removed. For example, there are more than 100 structures of His-tagged proteins in the Protein Data Bank. This indicates that the small His-tag

often does not interfere with correct protein folding. Additionally, tested activity results are listed on our protein web pages. If you have concerns about tags interfering with protein activity and there is no activity data online, please feel free to contact us for latest information at tech@medchemexpress.com.

11 What is the specific activity of your recombinant proteins? What is meant by a "unit" of protein activity?

The biological activity (ED_{50}) (or "unit") for each recombinant protein is available on the certificate of analysis. The ED_{50} is defined as the protein concentration at which the activity is 50% of the maximum response and is reported in ng/mL. This method of expressing activity should only be used for proteins whose dose-response curves are sigmoidal in shape. The formula for converting the activity as an ED_{50} to specific activity is:

$$Specific\ Activity\left(\frac{Units}{mg}\right)=\frac{10^6}{ED_{50}}\left(\frac{ng}{mL}\right)$$

Please note that MCE does not use the International Standard provided by WHO (National Institute for Biological Standards and control) for measuring recombinant protein activity. There is not a way to convert between these "International Units" and the ED_{50}. The best way to compare the activity of recombinant proteins from different sources is to do the same bioassay side-by-side using the same system.

12 Does the specific activity of recombinant proteins vary between lots?

Specific activity will vary for each lot and for the type of experiment that is done to validate it. It is recommended that you perform your own specific experimental validation to find out the optimal ED_{50} for your system.

13 I try to do an experiment with your protein, but the bioassay you use for determining activity is not the same as my application. Will my application work with your protein?

MCE products are used for many different purposes, so it would be impossible to predict every possible application. Our standard bioassay is used to confirm an accepted activity level for the product. Our proteins can be used at a broad concentration range, in many different applications, thus, it is the end user's responsibility to determine the concentrations that work best for their specific assays.

14 Do most proteins show cross-species activity?

Species cross-reactivity must be investigated individually for each product. Many human cytokines will produce a nice response in mouse cell lines, and many mouse proteins will show activity on human cells. Other proteins may have a lower specific activity when used in the opposite species.