

Instructions for Usage of Recombinant Protein

1 How many kinds of recombinant proteins does MCE offer?

- A. Classified by protein types: Cytokines, Growth Factors, Chemokines, Hormones, Neurotrophins, Enzymes & Inhibitors and Others.
- B. Classified by species: Human, Mouse, Rat, Rhesus Macaque, Procine, Canine, Bovine and Others.

2 How many protein expression systems does MCE offer?

MCE provides proteins expressed in E. coli, yeast, insect and mammalian 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. Bradford protein assay; b. BCA protein assay; c. Intensity measurement on the SDS-PAGE gel with a BSA standard curve.

4 What are the shipping conditions for recombinant proteins?

Most recombinant proteins are shipped at blue ice or 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. We recommend centrifuging the vial in a micro-centrifuge for 20-30 seconds before opening to drive any protein that may be lodged in the cap or on the side of the tube to the bottom of the vial. Our quality control procedures assure that each vial contains the correct amount of product.

7 How do you reconstitute lyophilized powder? What is an acceptable volume for recombinant protein reconstitution? What concentration is the recombinant protein upon reconstitution?

Please check the certificate of analysis included with your shipment for the exact instructions for reconstitution because not all products are reconstituted with the same conditions. In general, we recommend using sterile water for reconstitution. Add the recommended volume of sterile water to the vial, and gently shake it to solubilize the protein completely. Do not vortex.

Typically, 100 μ L is an acceptable reconstitution volume for our 10 μ g and 50 μ g vials of recombinant protein.

The concentration of recombinant protein is dependent on the reconstitution volume. For example, if reconstituting 10 µg of recombinant protein in 100 µL carrier protein solution, the concentration is 10 µg/100 µL or 100 µg/mL.

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

For longer term storage the protein solution should be stored with a carrier protein (e.g. 0.1% BSA or 0.1% HSA) in working aliquots and stored frozen at -20°C. Please keep in mind that every freeze/thaw cycle may cause some denaturation of the protein. The majority of recombinant proteins have a guaranteed shelf life of one year, unless indicated otherwise on the certificate of analysis. This guarantee is provided if they are kept under optimal storage conditions as stated on the certificate of analysis.

9 What is a carrier protein?

Carrier 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.

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.

- Protein tags are useful for protein purification.
- Tags are used for protein detection in Western blot or ELISA when the specific antibodies are not available.
- 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 (ED₅₀) (or "unit") for each recombinant protein is available on the certificate of analysis. The ED₅₀ 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 ED₅₀ to specific activity is:

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

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 ED₅₀. 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 a recombinant protein vary between lots?

Specific activity is unique for each lot and for the type of experiment that is done to validate it. We recommend that you perform your own specific experimental validation to find out the optimal ED₅₀ for your system.

13 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?

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.

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