

## RUO Cytokine FAQs

The relevant information relating to each product appears on the Certificate of Analysis (CoA) that is shipped with the product. Please read this information carefully to obtain instructions for reconstitution and storage. If, after reading the CoA, you need additional information, please review the following set of questions and answers, or call our Quality Assurance Department at QualityAssurance@PeperoTech.com or 800-436-9910 prompt number 4.

### 1. What should I know about the stability of your protein products?

Unless otherwise mentioned on the product information sheet, all of our products are formulated in such a manner that the lyophilized proteins are very stable at room temperature. However, we recommend storing lyophilized products at -20°C to -80°C.

For reconstituted solutions of most products, we recommend short-term storage at 4°C. For extended storage, the protein solution should be stored with a carrier protein (e.g. 0.1% BSA) in working aliquots and stored at -20°C to -80°C. Aliquots should be prepared to a concentration no lower than 1µg/ml, and contain at least 10µl, independent of concentration.

Please keep in mind that every freeze/thaw cycle may cause some denaturation of the protein; therefore, we do not recommend subjecting aliquots to more than a single freeze/thaw cycle.

### 2. What endotoxin level should be expected when purchasing PeperoTech proteins?

For PeperoTech's Animal-Free proteins, the endotoxin level is guaranteed to be less than 0.01 ng/µg of protein, or 0.1 EU/µg. For most of PeperoTech's non-Animal-Free proteins the endotoxin level is guaranteed to be less than 0.1 ng/µg of protein, or 1 EU/µg. However, for many proteins, the actual measured endotoxin values are consistently below these stated endotoxin levels. Please contact our Quality Assurance Department (QualityAssurance@PeperoTech.com) for more information.

### 3. Why can't I see the protein pellet in the vial?

Unlike many protein products available on the market, PeperoTech products are not formulated with carrier proteins or other additives (e.g., BSA, HSA, sucrose, etc.). As a result, the small amounts of protein can be deposited on the vial during lyophilization as a thin, and sometimes invisible, film. Before opening, we recommend centrifuging each vial in a microcentrifuge for 20-30 seconds to drive any protein that may be lodged in the cap or on the side to the bottom of the vial. Our quality control procedures assure that each vial contains the correct amount of product.

### 4. Which cytokines show cross-species activity?

With a few exceptions, most human cytokines are active on mouse cells. Many mouse cytokines are active on human cells, but may show lower specific activity than the corresponding human cytokine. The interferons, GM-CSF, IL-3, and IL-4 are known to be species-specific with very little, if any, activity on non-homologous cells. In contrast, the FGFs and neurotrophins are very highly conserved and show excellent activity on cells of other animal species.

### 5. What is the relationship between the specific activity expressed as an ED<sub>50</sub> and as units/mg?

While ED<sub>50</sub> is defined as the cytokine concentration at which activity is 50% of the maximum response, specific activity is defined as a measurement of reaction rate (i.e. activity) in relation to the amount or mass of a substance. Specific Activity Units should only be used as a method of expressing potency and should only be calculated for sigmoidal dose-dependent curves. The formula for converting activity expressed as an ED<sub>50</sub> in ng/ml to specific activity in units/mg is:

$$\frac{1 \times 10^6}{\text{ED}_{50} \text{ (ng/ml)}} = \text{specific activity (units/mg)}$$

### 6. What is the relationship between specific activity units and International Units of activity?

There is no direct correlation or calculation between specific activity unit and International Unit (IU) values.

International Units (IU) express a quantification of activity for the base amount of a substance in relation to an analogous reference standard with an internationally-accepted unit of biological potency (i.e. IU/ng) that has been assigned based on an International Collaborative Study conducted by the World Health Organization (WHO). WHO Reference Standards are made available by the National Institute for Biological Standards and Control (NIBSC).

Intended to simplify the comparison of activity of a substance obtained from different sources, IU measurements can vary as

comparison methods are rarely the same between sources. A true direct comparison requires standardized methods of analysis in order to guarantee comparability of the substance's activity in relation to its mass across sources.

**7. How does PeproTech obtain International Units of activity?**

Where possible, PeproTech obtains International Unit (IU) values through multiple side-by-side comparisons of our product(s) against the analogous WHO Reference Standard within our biological activity assay. Performing multiple comparison tests allows us to account for any outliers due to possible variations with the assay (e.g. product, handling, assay protocol, etc.). Using the results of these comparisons we can provide a reliable quantification of our product's activity in relation to the activity of the WHO Reference Standard.