

Converting ED₅₀ (ng/mL) to specific activity (units/mg)

Introduction

Cytokines are small signaling proteins that regulate a wide range of biological functions in cells, such as proliferation, differentiation, cell death, and cell migration. Many cytokines can exert different biological activities depending on the target cell and its environment, culture conditions, cofactors, or other synergistic effects. Therefore, the biological activity of a cytokine is routinely measured by its effect on a defined cell type under standardized conditions in a bioassay.

Determining the biological activity of cytokines

Bioassays are intended to measure the biological activity of specific growth factors or cytokines. In most cases, the bioassays are cell-based tests using different indicator cells, such as primary cells or cell lines. The cells are typically seeded on multi-well plates and incubated with a dilution series of the cytokine or growth factor. After a predefined incubation period, cell proliferation or cell death can be measured using a colorimetric assay. The most commonly used bioassays include the [cell proliferation assay](#), [chemotaxis assay](#), [cytokine production assay](#), and [cytotoxicity assay](#). The biological activity of a given cytokine is expressed as ED₅₀ (effective dose), which is the concentration of the cytokine that induces 50% of the maximum response. This method of expressing potency should only be used for a cytokine when its dose response curve is sigmoidal in shape (Figure 1).

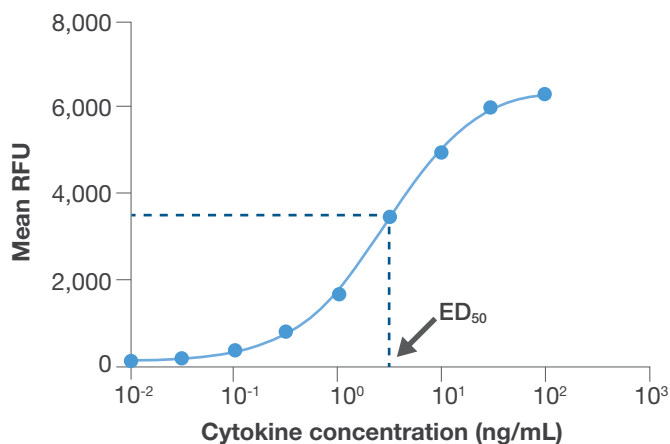


Figure 1. Example of a cell proliferation curve obtained using a recombinant cytokine.

Example calculation with Gibco™ Human IL-2 Recombinant Protein (Cat. No. PHC0021)

| Product Details | | |
|-------------------------|--|--|
| Size | 100 µg | |
| Species | Human | |
| Published Species | Human | |
| Expression System | E. coli | |
| Molecular weight | 15.5 kDa | |
| Class | Recombinant | |
| Type | Protein | |
| Purity | >95% by SDS-PAGE | |
| Endotoxin concentration | <0.1 ng/µg | |
| Activity | ED₅₀ < 0.5 ng/mL, determined by the dose-dependent proliferation of mouse CTLL-2 cells. | |
| Conjugate | Unconjugated | |
| Form | Lyophilized | |
| Purification | Size-exclusion - Gel filtration | |
| Contains | no preservative | |
| Storage conditions | 4° C | |

| Applications | Tested Dilution | Publications |
|----------------------------|-----------------|----------------|
| Western Blot (WB) | Assay-dependent | - |
| Immunohistochemistry (IHC) | Assay-dependent | - |
| ELISA (ELISA) | Assay-dependent | - |
| Functional Assay (FN) | Assay-dependent | 5 Publications |
| Control (Ctl) | Assay-dependent | - |

Product Specific Information
Carrier-Free

Reconstitution: Centrifuge the vial briefly, before opening to bring the contents to the bottom. Reconstitute the lyophilized protein in 100 mM acetic acid to 0.1-1.0 mg/mL to regain full activity. Make any further dilutions of the reconstituted protein in low endotoxin medium or buffered solution with FBS or tissue culture grade BSA.

Storage: Store the lyophilized protein at 2-8°C, preferably desiccated. Upon reconstitution, apportion into working aliquots and store at -20°C (not in a frost-free freezer). Avoid repeated freeze-thaw cycles.

Support 010: 1-800-898-0398
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Please note that while we do measure the specific activity of each lot of protein using a cell-based bioassay with Gibco™ cell culture medium, we encourage investigators to perform their own specific validation experiments to determine the optimal ED₅₀ for their specific system, as the exact values can vary for different cell types, cell culture media, and operators. The formula to convert ED₅₀ in ng/mL to specific activity (in units/mg) is:

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

Note: If the ED₅₀ is expressed as a range, use the midpoint of the range.

According to the example product information sheet, the ED₅₀ is <0.5 ng/mL, as determined by the dose-dependent proliferation of mouse CTLL-2 cells.

$$\frac{1 \times 10^6}{<0.5 \text{ ng/mL}} = >2 \times 10^6 \text{ units/mg}$$

Learn more at thermofisher.com/recombinantproteins

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