



Miltenyi Biotec



Recombinant cytokines and their biological activity

The biological activity of a recombinant cytokine (cytokine activity) is a measure for its effectiveness in inducing a particular biological response. Here, we describe how the cytokine activity is calculated and how cytokine activities can be calibrated to achieve comparable results.

Calculation of cytokine activity

In order to determine the cytokine activity, the cytokine concentration needed to induce half of the maximum response is identified based on the dose-response curve (fig. 1A). This concentration is called ED_{50} . The ED_{50} value can then be used to calculate the activity of the cytokine with a simple formula (fig. 1B).

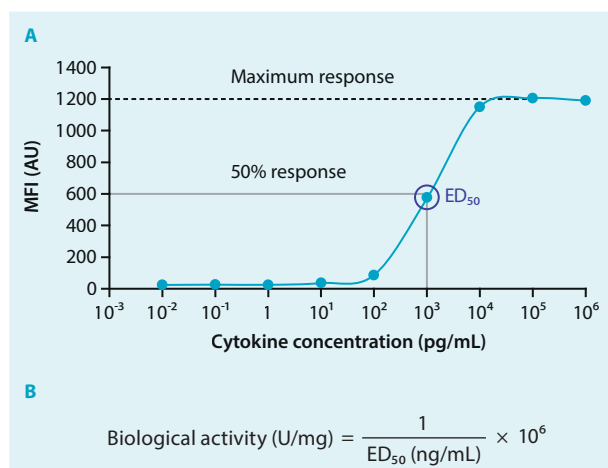


Figure 1: Determination of the ED_{50} value and cytokine activity. Based on the dose-response curve, the cytokine concentration which induces 50% of the maximum response (ED_{50}) can be determined (A). This ED_{50} value can be used to calculate the activity of the cytokine with a simple formula (B). In this example, the cytokine has an ED_{50} of 1×10^3 pg/mL (equals 1 ng/mL) and an activity of 1×10^6 U/mg.

The ED_{50} value and the biological activity are inversely correlated (fig. 2):

- Cytokines with a lower ED_{50} have a higher biological activity.
- Cytokines with a higher ED_{50} have a lower biological activity.

$ED_{50} = 1$ ng/mg

$$\text{Biological activity} = \frac{1}{1 \text{ ng/mg}} \times 10^6 = 1 \times 10^6 \text{ U/mg}$$

$ED_{50} = 10$ ng/mg

$$\text{Biological activity} = \frac{1}{10 \text{ ng/mg}} \times 10^6 = 1 \times 10^5 \text{ U/mg}$$

$ED_{50} = 0.1$ ng/mg

$$\text{Biological activity} = \frac{1}{0.1 \text{ ng/mg}} \times 10^6 = 1 \times 10^7 \text{ U/mg}$$

Figure 2: Exemplary cytokine activity calculations for different ED_{50} values. Cytokines with a lower ED_{50} have a higher biological activity and vice versa.

Calibration of cytokine activities

Bioassays used for the determination of cytokine activity result only in relative values. These values are not comparable between different assays, between different laboratories performing the same assay, or between the same assay performed in the same laboratory on different days.

To make the activities of cytokines comparable between different suppliers, the bioassay must be calibrated with a recognized standard reagent of known biological activity. The standard reagent is tested in the bioassay, which should be calibrated. The deviation of the measured cytokine activity from the known activity of the standard reagent can then be determined (fig. 3A) and applied to calibrate the activity values measured for cytokines with unknown activity (fig. 3B).

Standardized cytokine activities are calibrated values, which can be used to compare the activity between different cytokine products or batches, as long as these have also been calibrated using the same standard reagent.

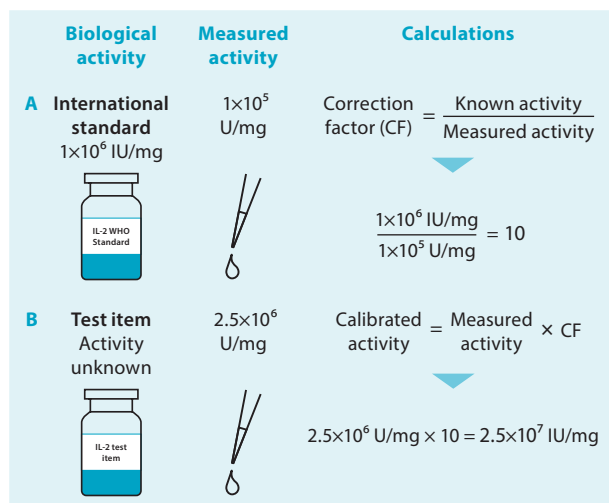


Figure 3: Exemplary cytokine calibration. In this example, a test vial of IL-2 is calibrated with the WHO standard for IL-2 (NIBSC code: 86/500). Based on the known activity of the international standard (1×10^6 IU/mg) and the measured biological activity of the international standard (1×10^5 U/mg), the calibration factor for this specific bioassay can be calculated with the shown formula (A). The applied bioassay underestimates the biological activity by a factor of 10. This correction factor can be applied to calibrate the activity values measured for the cytokine of unknown activity to achieve standardized activity values (B).

The difference between units and international units

Cytokine activities might be given in units (U) or international units (IU). While activities given in U might be calibrated with standard reagents of different reliability or not calibrated at all, activity values given in IU are always calibrated with WHO international standards.

The following list gives a detailed overview of different standard reagents used for cytokine calibration and table 1 summarizes the reliability for different designations for your cell culture application.

- WHO international standards (IS)¹ are manufactured according to WHO guidelines. They are extensively studied by multiple international laboratories and established by the WHO Expert Committee on Biological Standardization (ECBS). WHO international standards are available for many cytokines and are distributed by the National Institute for Biological Standards and Control (NIBSC).
- WHO reference reagents (WRR)² are standards that have not been tested extensively enough to be defined as "international standard". Nevertheless, these reagents can (and should) be used to calibrate bioassays.

- Internal standards (of a cytokine supplier) may be used when no international standard or reference reagents is available for a certain cytokine. The calibration with internal standards will allow the customer to compare different batches from one supplier. It will however not allow the customer to compare cytokine activities between different suppliers.
- If cytokines are sold without calibration, cytokine activities cannot be compared even between different batches of the same supplier. Thus, the activity for uncalibrated cytokines is usually not stated by the supplier.

Lot-specific cytokine activity of MACS® Cytokines

All MACS Premium-Grade and MACS GMP Cytokines are calibrated using NIBSC international standards or reference reagents whenever these are available. The specific activity for each cytokine lot is stated on the respective Certificate of Analysis (for MACS Premium-Grade Cytokines) or Product Quality Certificate (for MACS GMP Cytokines).

Calibration reagent	Measurement units	Reliability	Comparability
WHO international standard	IU	Highest	All cytokines with identical calibration (different suppliers & batches)
WHO reference reagent	U	High	
Internal standard	U	Medium	Different batches from same supplier
No calibration	U	Very low	No comparability

Table 1: Standard reagents used for cytokine calibration. Depending on which reagent was applied for the calibration of the cytokine activity, the activity is described in international units (IU) or units (U).

References:

1. https://www.nibsc.org/standardisation/international_standards.aspx
2. https://www.nibsc.org/standardisation/international_reference_reagents.aspx

GET OUR LISTS



Find a list of bioassays and calibration reagents used for calibration of our MACS Cytokines.

► [miltenyibiotec.com/cytokine-calibration](https://www.miltenyibiotec.com/cytokine-calibration)

Find our complete product lists here:

► [miltenyibiotec.com/cytokines](https://www.miltenyibiotec.com/cytokines)



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