

FAQ: Biological Activity of Cytokines

1 What *is* the biological activity of a cytokine, and what can we actually measure?

Cytokines can stimulate (or suppress) proliferation, differentiation, cell death, cell migration and have other effects on many cells. Many of those depend – to various degrees – on the target cell, environment of the target cell, culture conditions, cofactors or synergistic effects. Predicting - or quantifying – all these potential activities in one single numeric value is not possible. Therefore CellGenix measures one defined effect (e.g. stimulation of cell proliferation) on one defined target cell under standardized conditions (see also 4 – *why International units*).

2 How does CellGenix measure biological activity of Cytokines?

Every assay starts with cells from a defined Working Cell Bank stored in liquid nitrogen. Cells are thawed and cultivated in a defined medium until the needed cell number is reached.

Cells are then seeded on multiwell plates and incubated with a defined dilution series of the cytokine (usually all values determined in octuplicates). After a predefined incubation period, cell proliferation is measured by a colorimetric assay and the **ED₅₀** (“effective dose 50%” – the dose needed for 50% of maximum proliferative effect) calculated:

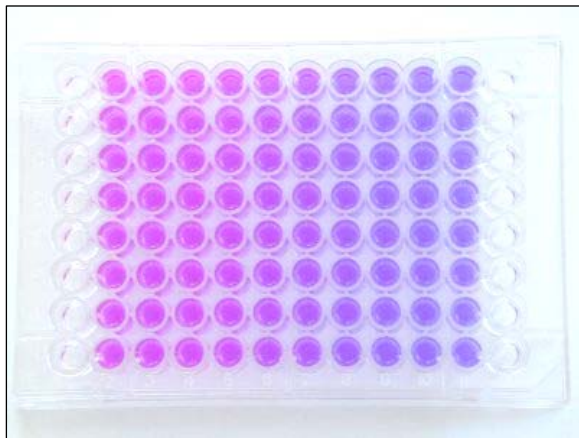


Fig.1: Colorimetric determination of cell proliferation after incubation with a cytokine dilution series. Metabolically active cells reduce a blue dye into a pink and fluorescent product that is a measure for cell number and viability.

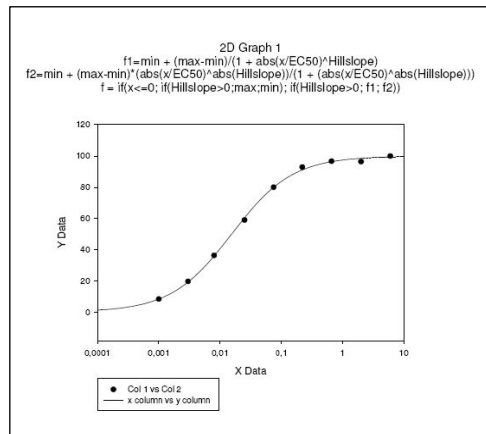


Fig. 2: Mathematical calculation of the ED50 value from mean values obtained by colorimetric assay.

The assays have been validated following ICH guidelines for each cytokine and are performed according to SOPs in our GMP facility using qualified equipment.

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3 How does the ED₅₀ translate into units?

By definition, one unit of activity is the amount of cytokine that has 50% of the maximal proliferative effect *per milliliter*.

Activity (units/mg) is calculated: $A \text{ (U/mg)} = 1 / ED_{50} \text{ (ng/ml)} * 10^6$ (1mg = 10⁶ng)

Example for GM-CSF: ED₅₀ = 0,1 ng/ml → $A = 1 / 0,1 * 10^6 = 1*10^7 \text{ U/mg}$

4 Why *International Units*?

The “unit” given as a result from cell proliferation assays does not itself carry any reliable or comparable dimension. It measures the proliferative effect of the product under *one* set of conditions, for *one* defined cell line, for an assay performed at *one* particular lab and with the reagents (especially cell culture media and serum) that are common at that lab.

Effects on other cells, cultivated under different conditions elsewhere can differ significantly. So when using “units” to compare activities of 2 batches of a cytokine, correctly they can only be used to compare two activities measured at the same lab, using the same batch of cells and the same batch of medium. In order to achieve some comparability, a defined standard needs to be used as a reference.

CellGenix thus, whenever an international standard exists, measures activity of its cytokines in relation to the international standard for that cytokine. Thus activities of our cytokines are normalized, using the defined WHO standard obtained from the NIBSC (National Institute for Biological Standards and Control), and given in *international units* per mg.

A sample of the NIBSC standard is used in parallel in *every* assay, and a correction factor is calculated for the relative activity of the CellGenix sample compared to the NIBSC sample.

5 How precise are the numbers?

As with any biological assay where a living organism is the “measuring instrument” these cell proliferation assays are prone to a certain amount of statistical variance. Viability and activity of the cells after thawing, quality of the cell culture medium and serum, temperature and incubation times are defined but will always differ to some small degree.

It is thus our policy, to determine biological activity for all GMP cytokines in duplicates and provide the mean value on our CoA.

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Therefore, customers should be aware that the statistical error in the given value will still be much greater than in other assays that do not rely on a biological system. As all measurements are also done on a logarithmic scale, a variance of up to +/- 20% should be taken into account.

6 Will the activity provided directly translate into our system?

The numeric value we provide on the CoA of the GMP products is the proliferative effect, relative to the international standard, on one defined cell line under CellGenix standard conditions.

Thus, while it is a good measure for the basic “functionality” of the product, it does not automatically mean that this *specific* activity translates 1:1 into every particular application. CellGenix generally advises its customers to use the given activity as a general benchmark, but perform an additional assay adjusted for their specific application when needed.

7 How do I compare activities of CellGenix Cytokines to those of other suppliers?

Unless the other supplier provides activity data in normalized *international units* as well, the various “in-house-units” cannot simply be compared with each other.

Even if activity data are normalized by using the same international standard they can only be compared if the same cell system is used because the same cytokine might have a higher or lower activity if another cell line is used.

The only condition, under which not-standardized units have the same dimension is when both samples are tested in the *same* assay. Even if the same cell line is used, there are still factors that will differ from lab to lab, and comparability of results will vary greatly.

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