Miltenyi Biotec



The reliability of stated cytokine activities

How reliable are the typical activity values given by suppliers? This is critical information when deciding which clinical-grade cytokines to purchase and determining how much is needed. Here, we examine this important question.

Note: We present normalized values in this application note to prevent the identification of products based on their activity values. Our aim is to sensitize users to the need for direct side-by-side testing rather than to promote a particular product.

The performance of clinical-grade cytokines can deviate significantly from the stated typical activity

We tested random lots of our MACS® GMP Cytokines (EGF,FGF-2, Flt3-Ligand, GM-CSF, IL-2, IL-4, IL-6, IL-7, IL-15, and Activin A) and clinical-grade cytokine products from two other suppliers side-by-side using industry-standard assays for activity determination.

The measured activities of our MACS GMP Cytokines did not deviate significantly from the stated typical activities (table 1 and fig. 1). In contrast, activities of the same cytokines from other suppliers deviated noticeably from the specified activities. In most cases, measured values were lower than indicated.

While a certain deviation of single batches from the stated typical activity is not unusual, it is desirable to keep this deviation as low as possible. For some of the cytokines, we found a more significant deviation than batch-to-batch variations can explain.

Cytokine	Miltenyi Biotec	Supplier 1	Supplier 2
EGF	126.7	321.4	250.0
FGF-2	111.1	125.0	26.7
Flt3-Ligand	103.9	90.0	35.0
GM-CSF	120.0	60.0	57.1
IL-2	100.0	40.5	14.1
IL-4	170.2	62.1	128.6
IL-6	100.0	136.4	48.0
IL-7	130.8	50.0	50.0
IL-15	100.0	2.2	50.0
Activin A	119.0	200.0	104.0

Table 1: The measured activities given in percent of the stated typical activities for the indicated cytokine products. Deviations exceeding 50% lower or higher than specified are indicated in red.

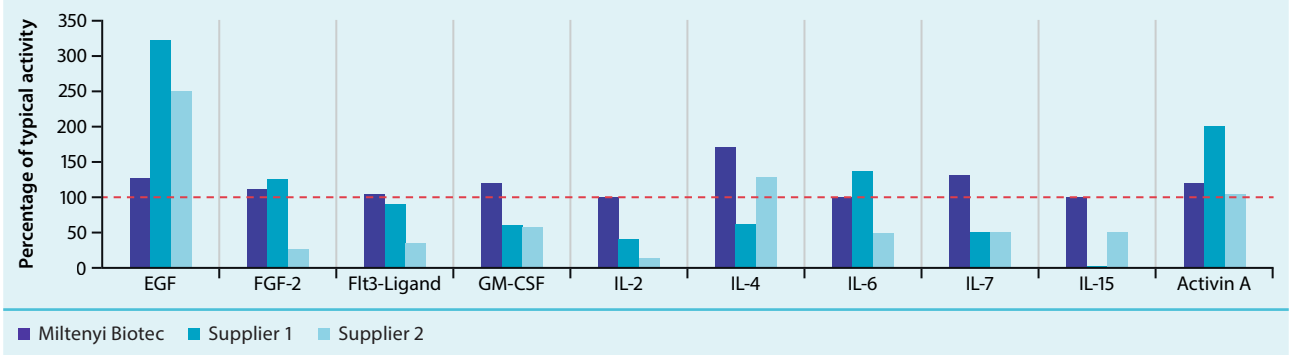


Figure 1: The deviation of the measured activity from the stated typical activity for the indicated cytokine products from Miltenyi Biotec, supplier 1, and supplier 2.

Deviations from the stated typical activities can be extremely significant

By far the largest deviation from the stated typical activity was observed for the IL-15 product from supplier 1. The real performance of the cytokine in a side-by-side measurement was only 2% of the stated typical activity (fig. 2).

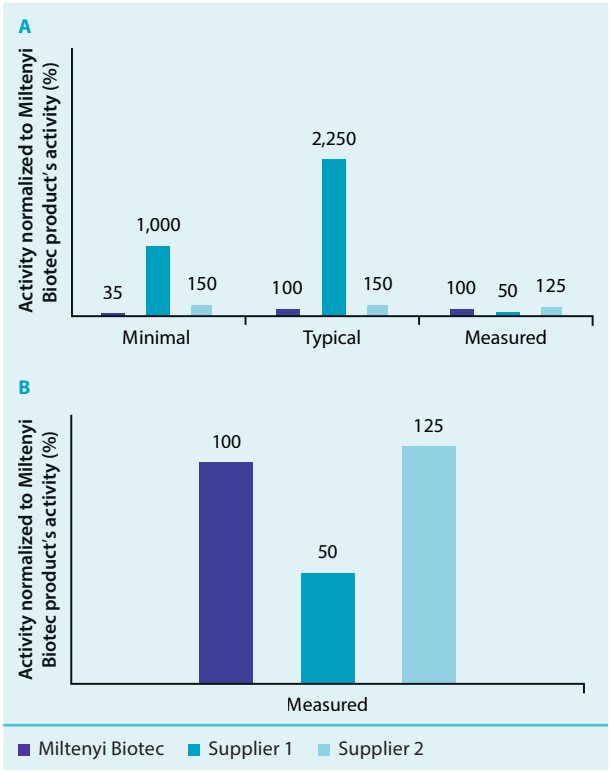


Figure 2: An overview of the stated (minimal, typical) and measured activities for IL-15 products. To prevent the identification of the other suppliers, all values are normalized to the activity measured for MACS GMP IL-15 (Miltenyi Biotec). (A) The stated (minimal, typical) and measured activities for IL-15 products from Miltenyi Biotec and the two other suppliers. (B) The measured IL-15 activities from A with a scale adjusted to the measured activities.

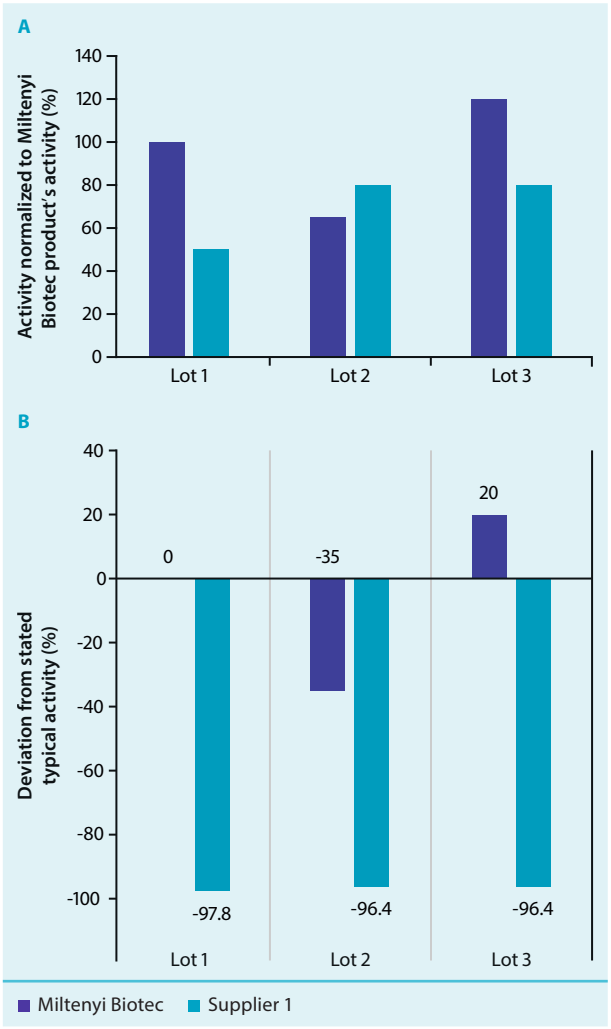


Figure 3: An overview of the measured activity of different IL-15 product lots from Miltenyi Biotec and supplier 1. To prevent the identification of the other suppliers, all values are normalized to the activity measured for MACS GMP IL-15 (Miltenyi Biotec). (A) The measured activities for 3 different lots of IL-15 products from Miltenyi Biotec and supplier 1. (B) The deviation of the measured IL-15 activities from the stated typical activities.

This is clearly the most extreme example of the deviations we report here. We confirmed our observations by testing additional batches of the respective IL-15 products and consistently obtained comparable results. The IL-15 product from supplier 1 performed slightly better in the follow-up tests (3.6% of indicated typical activity in both experiments), the overall deviation from the indicated activity was still very consistent with the results observed in our initial test (fig. 3).

Deviations from the stated typical activities are common

Various cytokine products have displayed similar deviations from the stated typical activities, although they are not always so pronounced. The impact of significant deviations on purchase decisions is obvious, but less pronounced deviations can also have economic consequences and should be considered.

Figure 4 depicts the results of a side-by-side comparison of a GM-CSF product. The performance of the products from suppliers 1 and 2 was ~40–50% below the stated typical activity. This is a completely different performance pattern than the stated typical activity suggested.

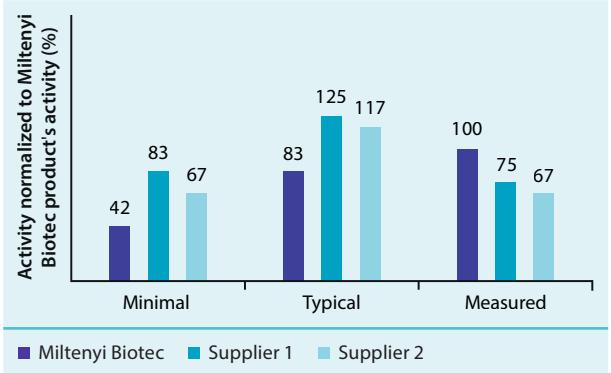


Figure 4: An overview of the stated (minimal, typical) and measured activities for GM-CSF products. To prevent the identification of the other suppliers, all values are normalized to the activity measured for MACS GMP GM-CSF (Miltenyi Biotec).

Figure 5 shows the results of the same type of comparison for FGF-2 products. The actual performance ranking was shown to be the opposite of that suggested by the stated typical activity: the cytokine product with the lowest stated minimal activity and intermediate stated typical activity had the highest measured activity.

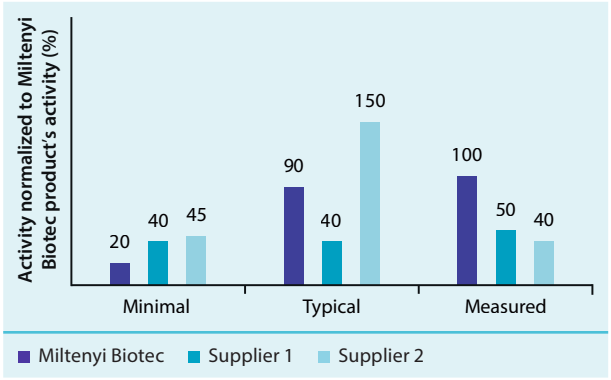


Figure 5: An overview of the stated (minimal, typical) and measured activities for FGF-2 products. To prevent the identification of the other suppliers, all values are normalized to the activity measured for MACS GMP FGF-2 (Miltenyi Biotec).

The consequences of inaccurate activity values

Ancillary materials used in the development or manufacturing of clinical-grade cellular products comprise a significant proportion of the overall cost. It is therefore essential that the activity values, which are the basis of any cost calculations, are accurate. Imprecise values (as were revealed in this study) can result in significant under or overestimations of the cost of raw materials and can have significant financial consequences.

A direct side-by-side comparison of cytokines from different vendors provides the clearest readout for performance in a specific biological assay. If side-by-side comparison is not possible, the precise lot-specific activity can be used as a measure of cytokine performance. We at Miltenyi Biotec calibrate the lot-specific activity with international standards. To provide accurate values, this calibration is performed in every single QC run and the lot-specific activity is clearly stated on the respective analysis certificate.

Why it is important to experimentally validate cytokine products

A recent survey conducted by Miltenyi Biotec showed that an estimated 75% of all laboratories and companies do not experimentally test clinical-grade cytokines prior to purchase. This implies that they are relying on the parameters specified by the supplier. As shown here, this can result in inaccuracies in the calculation of the amount of cytokine required.

A simple method to avoid basing calculations on potentially inaccurate values is to perform experimental side-by-side tests of cytokines from different suppliers. This establishes the accuracy of the activity and furthers the understanding of how these cytokines behave in the process they are intended for.

We therefore recommend comparing different cytokines by evaluating their performance in the intended experimental setup or application before choosing a specific cytokine product. The application note "Your guide to side-by-side cytokine comparison" offers detailed insight into the processes of side-by-side testing.

DOWNLOAD

Download our application note for side-by-side cytokine testing here:

▸ **miltenyibiotec.com/cytokine-testing**

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Miltenyi Biotec B.V. & Co. KG | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macsde@miltenyi.com | **www.miltenyibiotec.com**
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